

Proposal # 2001- <u>J-201</u> (Office Use Only)

PSP Cover Sheet (Attach to the front of each proposal)

Proposal Title: Biological Assessment of Green Sturgeon in the Sacramento-San Joaquin Watershed
 Applicant Name: Joseph J. Cech, Jr.
 Contact Name: Joseph J. Cech, Jr.
 Mailing Address: Dept. of Wildlife, Fish, and Conservation Biology, Univ. of Calif. Davis, CA 95616
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Amount of funding requested: \$ listed below

Some entities charge different costs dependent on the source of the funds. If it is different for state or federal funds list below.

State cost \$505,169 (@10%)Federal cost \$641,362 (@46.5-48%)**Cost share partners?**xxx Yes NoIdentify partners and amount contributed by each UC Davis, WFCB, \$12,739;UC Davis, Animal Science, \$12,739; CA Fish & Game, \$60,000**Indicate the Topic for which you are applying (check only one box).**

- | | |
|--|--|
| <input type="checkbox"/> Natural Flow Regimes | <input type="checkbox"/> Beyond the Riparian Corridor |
| <input type="checkbox"/> Nonnative Invasive Species | <input type="checkbox"/> Local Watershed Stewardship |
| <input type="checkbox"/> Channel Dynamics/Sediment Transport | <input type="checkbox"/> Environmental Education |
| <input type="checkbox"/> Flood Management | <input checked="" type="checkbox"/> Special Status Species Surveys and Studies |
| <input type="checkbox"/> Shallow Water Tidal/ Marsh Habitat | <input type="checkbox"/> Fishery Monitoring, Assessment and Research |
| <input type="checkbox"/> Contaminants | <input type="checkbox"/> Fish Screens |

What county or counties is the project located in? Sacramento, Yolo, Solano, Marin, Humboldt
Del Norte

What CALFED ecozone is the project located in? See attached list and indicate number. Be as specific as possible 1, 2, 3, 10, and 16.

Indicate the type of applicant (check only one box):

- | | |
|--|---|
| <input type="checkbox"/> State agency | <input type="checkbox"/> Federal agency |
| <input type="checkbox"/> Public/Non-profit joint venture | <input type="checkbox"/> Non-profit |
| <input type="checkbox"/> Local government/district | <input type="checkbox"/> Tribes |
| <input checked="" type="checkbox"/> University | <input type="checkbox"/> Private party |
| <input type="checkbox"/> Other: _____ | |

Indicate the primary species which the proposal addresses (check all that apply):

- | | |
|---|---|
| <input type="checkbox"/> San Joaquin and East-side Delta tributaries fall chinook salmon | <input type="checkbox"/> Spring-run chinook salmon |
| <input type="checkbox"/> Winter-run chinook salmon | <input type="checkbox"/> Fall-run chinook salmon |
| <input type="checkbox"/> Late-fall run chinook salmon | <input type="checkbox"/> Longfin smelt |
| <input type="checkbox"/> Delta smelt | <input type="checkbox"/> Steelhead trout |
| <input type="checkbox"/> Splittail | <input type="checkbox"/> Striped bass |
| <input checked="" type="checkbox"/> Green sturgeon | <input type="checkbox"/> A chinook species |
| <input checked="" type="checkbox"/> White Sturgeon | <input type="checkbox"/> All anadromous salmonids |
| <input type="checkbox"/> Waterfowl and Shorebirds | <input type="checkbox"/> American shad |
| <input type="checkbox"/> Migratory birds | |
| <input type="checkbox"/> Other listed T/E species: _____ | |

Indicate the type of project (check only one box):

- | | |
|---|---|
| <input checked="" type="checkbox"/> Research/Monitoring | <input type="checkbox"/> Watershed Planning |
| <input type="checkbox"/> Pilot/Demo Project | <input type="checkbox"/> Education |
| <input type="checkbox"/> Full-scale Implementation | |

Is this a next-phase of an ongoing project? Yes ☒ No ☐
Have you received funding from CALFED before? Yes ☒ No ☐

If yes, list project title and CALFED number Biological Assessment of Green Sturgeon in the Sacramento-San Joaquin Watershed

Have you received funding from CVPIA before? Yes ☐ No ☒

If yes, list CVPIA program providing funding, project title and CVPIA number (if applicable):

By signing below, the applicant declares the following:

- The truthfulness of all representations in their proposal;
- The individual signing the form is entitled to submit the application on behalf of the applicant (if the applicant is an entity or organization); and
- The person submitting the application has read and understood the conflict of interest and confidentiality discussion in the PSP (Section 2.4) and waives any and all rights to privacy and confidentiality of the proposal on behalf of the applicant, to the extent as provided in the Section.

JOSEPH J. CECH, JR.

Printed name of applicant

Joseph J. Cech Jr.
Signature of applicant



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410 Mrak Hall, One Shields Avenue
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CALFED Bay-Delta Program Office
1416 Ninth Street, Suite 1155
Sacramento, CA 95814

MRY 12 2000

Dear Colleague:

2001 Proposal Solicitation

Proposal Entitled "Biological Assessment of Green Sturgeon in the
Sacramento-San Joaquin Watershed"
Principal Investigator: Joseph J. Cech, Jr.

It is a pleasure to present for your consideration the referenced proposal.

Following the direction of "**Attachment D - Terms and Conditions for State Proposition 204 Funds**", this is to provide notification that the applicant takes exception to the following proposed "standard" clauses:

Section 6. Substitution
Section 9. Rights in Data
Section 11. Indemnification, and
Standard Clauses-Insurance Requirements - DWR

In order to bring the above provisions into conformity with the University of California Policy, we reserve the right to discuss with the aim of properly modifying these sections, should this proposal result in a subsequent award.

Please contact the principal investigator for scientific information. Administrative questions may be directed to my assistant, **Ms. Petrina Ho**, or me by telephone, facsimile or electronic mail at the numbers cited above. Furthermore, correspondence pertaining to this proposal and any subsequent award should be sent to the Office of Research and to the principal investigator.

Sincerely,


Sandra M. Dowdy
Contracts & Grants Analyst

Enclosures
Cc: J. Cech

Proposal to:

Name CALFED Bay/Delta Program
Address 1416 Ninth Street, Suite 1155
Sacramento, CA95814

Submitting Organization

THE REGENTS OF THE UNIVERSITY OF CALIFORNIA
UNIVERSITY OF CALIFORNIA
ONE SHIELDS AVENUE
DAVIS, CALIFORNIA 95616

Title of Proposed Research:

Biological Assessment of Green Sturgeon in the
Sacramento-San Joaquin Watershed

Total Amount Requested:

\$505,169 (@10%)
\$641,362 (@46.5-48%)

Proposed Duration

24 months

Desired Starting Date:

4-01-01

Principal Investigator/

Co-Investigator(s):

Department:

Phone Number:

J. J. Cech, Jr., WFCB, (530) 752-3103/ S. I. Doroshov, An. Sci., (530) 752-7603; B. P. May, An. Sci., (530) 754-8123; A. P. Klimley, BML, (707) 875-2055; C. E. Crocker, SFSU, NA; D. W. Kohlhorst, CDFG,

Checks Made Payable to: (209) 948-7080; R. G. Schaffter, CDFG, (209) 948-7081

The Regents of the University of California

Send Checks to:

CASHIER'S OFFICE
UNIVERSITY OF CALIFORNIA
ONE SHIELDS AVENUE
DAVIS, CA 95616

Send Award Notice to:

OFFICE OF RESEARCH
UNIVERSITY OF CALIFORNIA
ONE SHIELDS AVENUE
DAVIS, CA 95616
(530) 752-2075

Approvals:

Joseph A. Cech Jr. 5-12-00

Principal Investigator

Date

Co-Investigator

Date

Co-Investigator

Date

Department Chair

Date

MAY 12 2000

Dean, College/School

Date

Sandra M. Dwyer
Official Signing for Organization

PD:/kirkman.fcm/proposal

B. EXECUTIVE SUMMARY

BIOLOGICAL ASSESSMENT OF GREEN STURGEON IN THE SACRAMENTO-SAN JOAQUIN WATERSHED

Amount Requested: **\$505,169 (@10% overhead)** or **\$641,362 (@46.5, 48% overhead)**

Applicant: Joseph J. Cech, Jr., Department of Wildlife, Fish, and Conservation Biology, University of California, Davis, 1 Shields Ave., Davis, CA 95616; ph: 530-752-3103, FAX: 530-752-4154, e-mail: jjcech@ucdavis.edu

Participants and Collaborators: **S.I. Doroshov** (UCD), **B.P. May** (UCD), **A.P. Klimley** (UCD, BML), **C.E. Crocker** (SFSU), **D.W. Kohlhorst** (CDFG), **R.G. Schaffter** (CDFG)

The green sturgeon (GS, *Acipenser medirostris*) is an anadromous, native fish that occurs in low numbers in our Bay/Delta system. It is classified as a CALFED At-Risk Species (Priority Group I), but very little is known about its life history. Basic GS life history information is critical to this species' protection, and our project's targeted research focus is on the biological characteristics of this species and its habitats towards their eventual restoration. During the first two phases of our coordinated UC Davis-CDFG team effort, we are: 1. resolving some key areas of scientific uncertainty concerning GS' temperature-related food consumption, metabolic, growth and developmental responses; 2. developing molecular markers to differentiate GS from white sturgeon (WS) at early life stages and to differentiate GS populations in the Sacramento-San Joaquin watershed from other GS populations; and 3. searching Feather River habitats for evidence of GS spawning (Appendix 1). We have made technical presentations of our results to date at two workshops (Davis, CA, and Weitchpec, CA) and at the annual meeting of the California-Nevada Chapter of the American Fisheries Society (Ventura, CA) in the past few months. One manuscript from the project has been accepted for publication in the *Transactions of the American Fisheries Society* and another submitted to the same journal.

During the proposed Phase 3, we plan to continue addressing key areas of scientific uncertainty about GS, including new studies on subadults' and adults' movements, to refine our conceptual model and improve management of this species and its population(s) in the lower Sacramento-San Joaquin watershed. Phase 3 has five objectives: 1. determine juvenile GS temperature, dissolved oxygen, and salinity tolerance limits (using laboratory tanks) and behavioral tendencies (using annular gradient tank); swimming performance (using swimming flumes); and stress responses (using laboratory tanks & assays, Task 1); 2. characterize GS gonadal sex differentiation, stages of gametogenesis, fecundity and egg size in relation to age and body size (using samples from captive and wild populations); investigate GS chorion function and substrate attachment in fertilized eggs (using histochemical staining for glycoproteins and scanning electron microscopy); and determine optimal temperature ranges for GS larval development, growth, and survival (using laboratory tanks, Task 2); 3. use genetic techniques to accurately identify GS from white sturgeon (WS, *A. transmontanus*) at early life stages and examine the uniqueness of GS stocks (using nuclear microsatellite and mitochondrial DNA markers, Task 3); 4. determine the directions and rates of adult/subadult GS movements in San Pablo Bay, Yolo Bypass Toe Drain, and Klamath River (using ultrasonic and radio telemetry, Task 4); and 5. assess the distribution and abundance of GS in San Pablo Bay (using trammel net samples) and provide GS specimens for UC Davis (including Bodega Marine Laboratory, BML) and San Francisco State University (SFSU) scientists conducting the studies outlined in the first four objectives (Task 5). This project will provide valuable information to decision-makers using adaptive management to resolve scientific uncertainties in our GS life history conceptual model (Figure 1) and assist in GS recovery, a specific ERP (Vol. 1, WS and GS, pp. 146-148; Vol. 2, WS and GS, p. 276) and AFRP (pp. 40-41, 70-71, and 95) objective of CALFED and CVPIA.

C. PROJECT DESCRIPTION (8 pages excluding figures and tables)

1. Statement of the Problem

The green sturgeon (*GS*, *Acipenser medirostris*) is an anadromous, native fish that occurs in low numbers in our Bay/Delta system (Moyle 1976). It is classified as a CALFED At-Risk Species (Priority Group I), but very little is known about its life history. Basic GS life history information is critical to this species' protection. How does GS food consumption rate, metabolic rate, and growth rate respond to environmental temperature or food resource availability? Which temperatures, salinities, and dissolved oxygen concentrations do the juveniles GS behaviorally select and what are its tolerance limits to these variables? What do these "preferences" and tolerance limits of the developing juveniles indicate about emigration rates from the rivers to the estuary and ocean? Environmental requirements data are quantitatively linked via bioenergetic models (Jobling 1994) that allow predictions of physiological shortcomings (e.g., reduced growth, reproduction, survival) associated with environmental stresses (measured by tolerance limits and hormonal responses) that lead to populational declines (Wedemeyer et al. 1990). Stress negatively impacts the health, growth and reproductive success of fish. Stress responses (e.g., to temperature changes, low water quality) can lower reproductive success and, may account for unexplained failure of populations to reproduce normally. However, studies to test this hypothesis are not possible until a fundamental understanding of the GS' stress response is developed. In addition, little information exists on the GS' reproductive characteristics, such as gonadal development, age and body size at sexual maturity, fecundity, egg size, and developmental rates of eggs and larvae. How do these characteristics compare with the much better-studied WS in our Sacramento-San Joaquin Bay-Delta system? Further, proper management of any species in a mixed-stock fishery depends in part on the proper identification of all life history stages (for accurate determination of population size and sustainable yield), identification of existing stock structure, and an accurate estimate of each stock's contribution to the fishery. GS and WS currently support a highly exploited mixed-stock fishery (Love, 1996). Finally, we know very little about the GS' movements, including the important spawning-related migrations along the ocean-estuary-river path. Do subadults move from the ocean into San Pablo Bay during the late summer to feed? How long do migrating, adult GS remain in the estuary? What are spawning GS' locations and movements in the Klamath River (where spawning GS can be reliably accessed through Yurok Tribe cooperation), and what can these data indicate regarding preferred GS spawning sites and these sites' physical characteristics (e.g., in Feather River)? Disturbingly, during "wet" years, sturgeon are being stranded at the Fremont Weir (unable to enter the Sacramento River channel after apparently migrating up the Yolo Bypass), and information is vitally needed on GS (and WS) movements and swimming performance to assess proposed solutions to this stranding problem.

American GS are known to spawn in the Sacramento and Klamath Rivers (Moyle et al., 1994), and the adults are present in the lower reaches of the Columbia and Fraser Rivers (Houston, 1988). Artyukhin and Andronov (1990) described spawning runs of the Asian GS (considered the same species *A. medirostris* Ayres or, as subspecies *A. medirostris mikadoi* Hilgendorf) in the Tummin River (Sakhalin Island) and succeeded in the artificial spawning of two females. However, they provided no detailed descriptions of early GS development. Our CALFED project's (Project No. 98-C15 [B81738]) current activities (Phases 1 and 2) are concentrating on measuring GS' food consumption, metabolic, and growth responses; determining its spawning, egg fertility, and larval survival characteristics; developing genetic techniques for distinguishing GS (from WS and among GS stocks), and searching Feather River habitats for evidence of GS spawning (see Appendix 1 for detailed review). Through our Phase 1 and 2 activities, we collected samples of gonads and fin rays from 30 wild-caught adults (Klamath River) and 14 subadults (San Pablo Bay) to examine gonadal

development in relation to age, body size, and sex. We also conducted the first artificial spawning of North American GS (May 1999) on the Klamath River in collaboration with the Yurok Tribe, reared GS juveniles at UC Davis campus, photographed the developmental stages of the embryos and larvae, and are preparing a manuscript describing their normal development. The resulting juvenile GS were used in studies of ration size and temperature effects on food consumption rate, growth rate, and food conversion efficiency. Studies were also conducted on GS juveniles' metabolic (oxygen consumption) rates, and preliminary data were collected on the developing GS' stress responses. Due to Prof. G. Moberg's untimely death (**August**, 1999), Prof. Moberg's GS stress research activities (and graduate student, Scott Lankford) were transferred to J. Cech's laboratory, and the experiments resumed, albeit with a "no-cost" extension to Phase 1 (delaying the Phase 1 *final* report until 10-1-00). Young GS are difficult to morphologically distinguish from sympatric WS. However, the development of a mitochondrial DNA marker, which uses a specific restriction enzyme site, yields a single DNA sequence in WS and two smaller sequences in GS. Further, amplified fragment length polymorphism (**AFLP**) differences between GS and WS were used to prepare primers from DNA sequences that show a seven base-pair deletion in GS, compared with WS. Finally, possible Feather River spawning sites are being sampled using artificial substrates at several sites between Shanghai Bend (Rkm 41) and Thermalito outfall (Rkm 95) that have depth, velocity and substrate characteristics typical of spawning sites of other sturgeon. Also, larval nets (Kohlhorst 1976) are being fished at locations between the southern end of the Oroville Wildlife area (Rkm 87) and the Highway 99 bridge near Nicklaus (Rkm 15). Throughout the spawning season, flow, temperature, and substrate type are also being measured at several locations.

Phase 3 work (outlined in this proposal) has five objectives: **1.** determine juvenile GS' temperature, dissolved oxygen, and salinity tolerance limits (using laboratory tanks/assays) and behavioral tendencies (using annular gradient tank); swimming performance (using swimming flumes); and stress responses (using laboratory tanks/assays, Task 1); **2.** characterize GS gonadal sex differentiation, stages of gametogenesis, fecundity and egg size in relation to age and body size (using samples from captive and wild populations); investigate GS chorion function and substrate attachment in fertilized eggs (using histochemical staining for glycoproteins and scanning electron microscopy); and determine optimal temperature ranges for GS larval development, growth, and survival (using laboratory tanks, Task 2); **3.** develop genetic techniques to accurately identify GS at all life history stages and examine the uniqueness of GS stocks (using nuclear microsatellite and mitochondrial DNA markers, Task 3); **4.** determine the directions and rates of movement of adult/subadult GS in San Pablo Bay, the Yolo Bypass Toe Drain, and the Klamath River and the relative importance of temperature, salinity, and water current direction (using ultrasonic and **radio** telemetry, Task 4); and **5.** assess the distribution and abundance of GS in San Pablo Bay (using trammel net samples) and provide GS for Tasks 1-4 (Task 5). Fisheries biologists from UC Davis, CDFG, BML, SFSU, and the Yurok Tribe will join in this collaborative effort to provide valuable information for adaptive management approaches to increase our Bay-Delta GS stocks through resolution of current scientific uncertainties in our GS life history conceptual model (Figure 1).

Conceptual Model

Figure 1 shows our conceptual model linking the GS' life history in the Sacramento-San Joaquin watershed ecosystems (river, including bypass, and estuary) to the Pacific Ocean. The rectangles represent the various ecosystems that the anadromous GS occupy at various life stages, but many scientific uncertainties exist regarding their spatio-temporal pattern(s) and movements (arrows) in this system. The questions raised and samples/experiments started and proposed (see

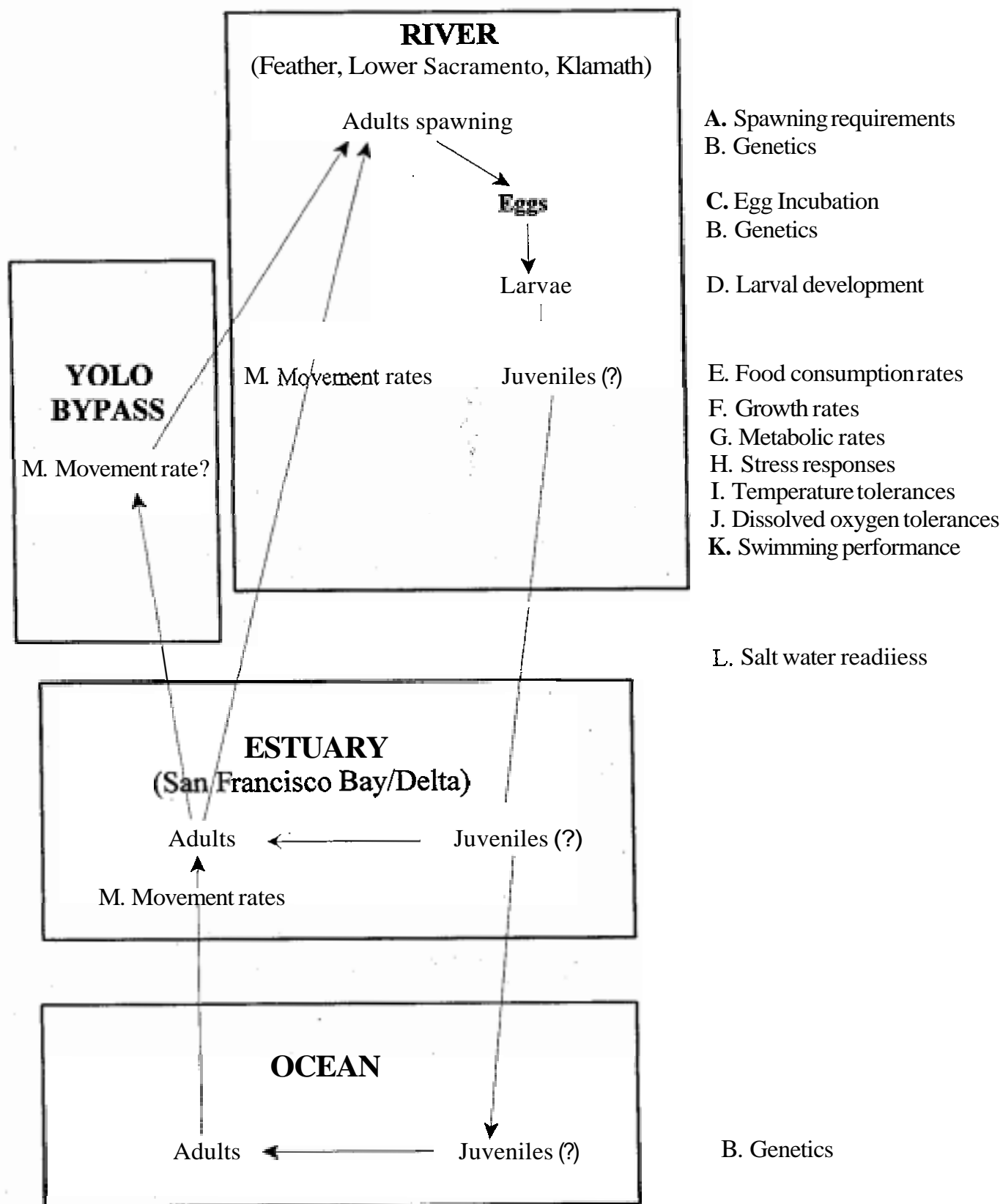


Figure 1. Conceptual model, with (lettered) targeted research elements, for GS distribution and movements in the Sacramento-San Joaquin watershed and linked ecosystems.

Statement of the Problem, above) are shown as the question marks (regarding distribution of the juveniles) and (lettered) approaches listed near the various life stages. Resolving more of the key spatio-temporal patterns (i.e., putting approximate dates [times of year] and/or fish ages with life stages in the various ecosystem components) will remove these uncertainties and provide valuable information to decision-makers using adaptive management to assist Sacramento-San Joaquin watershed GS populational recovery, a specific EFP (Vol. 1, WS and GS, pp. 146-148; Vol. 2, WS and GS, p. 276) and AFRP (pp. 40-41, 70-71, and 95) objective of CALFED and CVPIA.

Hypotheses Being Tested

The five project tasks ~~will~~ test several hypotheses to help achieve the CALFED Ecosystem Restoration Goal (#1) for GS: recovery towards large, self-sustaining populations, minimizing the need for future listing as ~~an~~ endangered species (CALFED Ecosystem Restoration Program Plan, Strategic Plan for Ecosystem Restoration, p. 21). Hypotheses letters refer to lettered approaches in conceptual model (Figure 1). **A.** GS spawning requirements are dependent on season (spring), river flow (temperature and current velocity), and spawning substrate (bedrock or gravel) (Tasks 2, 5). **B.** GS account for <10% of the sturgeon egg production in the Feather/Sacramento Rivers. This hypothesis is currently being tested in Phases 1 and 2 (Task 3). Feather/Sacramento River GS are being caught in the Oregon and Washington ocean fisheries (Task 3). **C.** GS egg incubation ~~may~~ occur in the crevices of the rocks or in the gravel, as suggested by low adhesive properties of egg chorion (Task 2). **D.** Optimal river temperature range for the GS larval development, growth, and survival is most likely within 14-20°C (Task 2). **E.** GS food consumption rates significantly increase with increased temperature (to some maximum within its temperature tolerance range) and ration **size**. This hypothesis is currently being tested in Phases 1 and 2. **F.** GS growth rates significantly increase with increased temperature (to some maximum within its temperature tolerance range) and ration size (currently being tested). **G.** GS metabolic rates ~~will~~ significantly increase with increased temperature (to some maximum within its temperature tolerance range, currently being tested). **H.** GS stress responses are activated at a significantly earlier age than those of WS (research underway), but are significantly more sensitive to increased temperature and decreased dissolved oxygen stressors than to increased salinity stressors (Task 1). **I.** GS (upper) temperature tolerance limits significantly decrease as the juvenile fish develop during their first year (Task 1). **J.** GS (lower) dissolved oxygen tolerance limits significantly increase as the juvenile fish develop during their first year (Task 1). **K.** GS swimming performance, in terms of critical swimming velocity (cm/s, Brett 1964) significantly increases as the fish grow in length, and increases as temperature increases (to some maximum within its temperature tolerance range, Task 1). **L.** GS become more salt water tolerant (i.e., tolerate hypertonic environments) as they develop during their first year, and GS are more salt water tolerant than WS at the same size (Task 1). **M.** GS movement is more directed (and movement rate is significantly greater) with the water current axis (strong rheotaxis, either positive or negative) in rivers than its direction and movement rate toward the salinity gradient axis in San Pablo Bay (Task 4). GS movement directions are correlated (non-random distribution) with temperature (Task 4). GS move upstream (only) in the Yolo Bypass (towards their putative spawning sites in the Feather and Sacramento Rivers) at a rate that is non-significantly different than that shown in the Klamath River (where pre-spawning adults can be reliably accessed, Task 4). **Our** coordinated approach of testing these hypotheses **will** resolve key scientific uncertainties in the conceptual model (Figure 1) and will significantly assist in GS recovery, a specific ERP (Vol. 1, WS and GS, pp. 146-148; Vol. 2, WS and GS, p. 276) and AFRP (pp. 40-41, 70-71, and 95) objective of CALFED and CVPIA.

Adaptive Management

The various samples and experiments that comprise this project should systematically remove the scientific uncertainties shown in the conceptual model. Also, as data are collected and analyzed, more quantitative hypotheses can be posed to more accurately determine the spatio-temporal patterns of GS distribution and abundance in the Sacramento-San Joaquin watershed ecosystems. For example, the salt water tolerance (phase 3) and the growth rate responses to temperature experiments (Phases 1 and 2) on developing juvenile GS will better define their environmental (niche) requirements and indicate their emigratory timing capabilities (and, therefore, likely distribution) in the system for a particular year (and its river temperature regime). These estimates can be checked with GS samples from ongoing field sampling efforts (e.g., CDFG real-time monitoring program). Resolution of these uncertainties requires rigorous research approaches (e.g., where statistically defensible, collected data are used to evaluate and refine the conceptual model) and cannot be adequately addressed through pilot or implementation projects.

Educational Objectives

Although the project is targeted research, several UC Davis and SFSU graduate and undergraduate students will be part of the research team to reduce scientific uncertainties concerning GS life history. Regular reports at workshops, meetings, and in the IEP Newsletter, and peer-reviewed publications will help disseminate results to the interested public and to professionals. Dr. Peter Klimley is based at the BML, bringing BML student exposure, and Dr. Carlos Crocker brings SFSU students (as well as increased ethnic diversity) to the project.

2. Proposed Scope of Work

Location

Project field locations will be in San Pablo Bay, the Sacramento River/Yolo Bypass system, and Klamath River. Although the Klamath is not technically part of the Sacramento-San Joaquin watershed, river tracking of GS is planned there because it is an established GS spawning site and we enjoy an excellent working relationship with our Yurok Tribe colleagues. Some Task 4, GS holding is planned for SFSU, and various laboratory studies are planned at UC Davis.

Approach

Task 1. GS Environmental Tolerance Limits and Behavioral Tendencies. Stress Responses and Swimming Performance: Temperature, salinity, dissolved oxygen, and water current play important roles in the development and survival of young fish. Using juveniles (several life stages: post-larvae through 1-kg wet weight GS) from UC Davis GS spawnings (see Task 2), tolerance limits of temperature (critical thermal maxima and minima, Becker and Genoway 1979), dissolved oxygen, and salinity (Young and Cech 1996) will be determined (loss of equilibrium endpoint). Loss of equilibrium in fish indicates physical disorganization due to the experimental variable and loss of the fish's ability to escape from conditions leading to its death (Becker and Genoway 1979). A horizontal, annular temperature gradient tank (1.0 m diameter) with a telethermometer/probes array in the swimming path and a video camera/monitor system will be used for GS' (acclimated to 11, 15, or 19°C) behavioral tendencies (temperature, dissolved oxygen, and salinity selection) experiments. GS swimming performance (critical swimming velocities, U_{crit}) will be determined at 11, 15, and 19°C using a modified Brett-type recirculating water flume (juveniles) or open-topped flume (subadults and adults) incorporating variable-speed motors (Brett 1964, Beamish 1978, Young and Cech 1996).

To characterize the GS' interrenal (stress) response, individual fish **will** be anesthetized with MS 222, and fitted with an indwelling cannula (caudal vein) for subsequent blood sampling. Blood samples **will** be analyzed for cortisol, testosterone, 17α , 20β DH-pregnenolone and estradiol using radioimmunoassay (Moberg et al., 1995; Faulkner and Moberg, 1997). Plasma cortisol (Daly et al., 1999), corticosterone, glucose, and lactate levels **will** be measured in response to an air emersion stressor and an ACTH infusion in cannulated fish (Belanger et al., in review). In addition, we **will** purify and quantify glucocorticoid receptor populations in specific target tissues using molecular techniques (Ausebel, 1987). These measurements **will** yield a hormone to receptor ratio that will indicate the GS' ability to respond to chronic vs. acute stressors, as checked by changes in metabolic (oxygen consumption rates) and swimming performance (U_{crit}) that accompany the effects of acute and chronic stressor regimes. If the proposed model is supported, it would provide evidence for a physiological trait that could be contributing to GS' low population size. Appropriate statistical models (ANOVA, Kruskal-Wallis, and post-hoc tests) **will** be used to compare means.

Task 2. Reproductive Characteristics of Wild GS, and Temperature Influences on Larval Development:

Based on the successful collaboration with the Yurok Tribe during Phase 1, we **will** continue collecting data and tissue samples from wild GS (Klamath and Sacramento Rivers) for fecundity, egg size, stage of oocyte maturity, and gonadosomatic index (in males and females) data of migratory GS spawners. These data and finray samples **will** be used to correlate reproductive characteristics with age and body size of sampled fish, using techniques and methods described in our original proposal. In addition, we are rearing and sampling (3-mo intervals) the offspring of artificially spawned (May 1999) GS to obtain information on age and size at gonadal sex differentiation, sex ratio, and the early phases of gametogenesis in male and female.

Our preliminary observations on fertilized eggs of two spawned GS females indicate that their egg chorion has unusually weak adhesive properties compared with WS eggs and some other species. The egg chorion structure and adhesive properties vary among sturgeons and are believed to correlate with spawning substrate and river current (Vorobyeva and Markov, 1999). We **will** examine GS' chorion structure (histochemical staining for glycoproteins and scanning electron microscopy) and the jelly formation process (Cherr and Clark, 1985). We **will** also attempt to quantify the GS eggs' relative adhesivity (freshly fertilized eggs **will** be allowed to adhere to glass and rock substrates and tested for the strength of attachment in the lab shaker over a post-fertilization time range) and the substrate attachment mode, compared to WS' eggs. Investigation of chorion properties is important because GS spawning grounds' characteristics may more resemble certain rivers and streams and act as a reproductive isolation mechanism between sympatric GS and WS (both species have similar cell DNA content and approximately similar chromosome numbers, [Van Eenennaam et al., 1999] and therefore may produce fertile hybrids).

Sacramento and Klamath Rivers' temperature regimes are affected by water projects and vary in the spring and summer. These regimes may be outside the optimal range for the GS' larval nursery phase. Because larval survival and growth through metamorphosis may determine GS recruitment and seaward migration time, optimal temperature ranges for larval GS development and growth **will** be studied. GS artificial spawning and larval rearing techniques that are being established (Van Eenennaam et al., submitted) **will** provide "yolk sac" larvae for rearing (to metamorphosis) at 6 temperature levels (range: 12 – 26°C) for determining temperature-related effects on survival, rates of abnormal development, growth (dry weight), and weight-

length relationship (Wang et al. 1987). The experimental design will include **5** replicate tanks in each temperature level and the data will be analyzed using appropriate ANOVA and regression models.

Task 3, Genetic Analysis: In Phase 1 of this project nuclear and mitochondrial DNA markers were developed to distinguish between early life history stages of GS and WS. In addition, nuclear microsatellite markers were developed that could characterize genetic variation within and between GS populations in California and Oregon. In Phase 2 work (currently underway), these markers are being used to investigate stock structure in GS populations from the Sacramento-San Joaquin Delta, Klamath River, and Rogue River (Oregon) watersheds and to identify and quantify numbers of GS vs. WS eggs and fry captured by CDFG personnel. Whole embryos or fin samples will be collected and stored in 95% ethanol. Fin sections are taken with utility razor blades and these are exchanged and dissection boards rinsed with water and 70% ethanol between individuals. Genomic DNA will be extracted using the CTAB phenol/chloroform protocol (Saghai-Marooft et al. 1984, Doyle and Doyle 1987, Grewe et al., 1993). Cooperative links with various investigators in California, Oregon, and Washington have been established, and Phase 3 genetic investigations will continue to use the species-specific markers (e.g., AFLP, described in original proposal) to identify young GS life history stages collected by our cooperators in various watersheds. This comparison is essential to determine the uniqueness of our Sacramento-San Joaquin watershed GS. We will use microsatellite markers to estimate the number of parents contributing to the spawning of these young GS in various watersheds, identify GS populations present in the mixed-stock fishery at the Columbia River mouth and off the Oregon coast, and estimate each stock's contribution to the total catch, each year over the two years.

Task 4, Telemetry:

Future plans to restore GS populations must be formulated with knowledge of the spatial distribution of the species as well as its environmental requirements. This task will focus on describing the subadult and adult GS' movements and natural habitats by tagging and tracking individuals with (including temperature-sensing) ultrasonic and radio transmitters within San Pablo Bay (and associated Delta waters) and the Klamath River, especially regarding relevant habitat characteristics. GS will be caught in trammel nets set in San Pablo Bay during late summer and fall. (see Task 5). The decrease in rate of capture between September and October and later re-capture of GS with fin-tags north of San Francisco after 3 mo to >1 year indicate that adults leave the bay in late fall and migrate along the coast northward toward the coast of Oregon (D. Kohlhorst, pers. com.). CDFG will begin fishing for GS in August during the grant period to capture individuals early during their outward migration so that we may track them in the bay (see Task 5). After we measure captured GS lengths, we will place transmitters on 10 adult GS during each year using two methods: 1. insertion of internal tags into the peritoneum of five GS, closure of incision (sutures), maintenance of the individuals in captivity (SFSU tanks with continuous bay water flows) until the insertion wound heals (ca. 3 d), and release of the GS at the site of capture; and 2. attachment (stainless steel wire) of external tags to GS and immediate release. Tagged GS will be tracked continuously, exchanging tracking crews at 12-hr intervals, for periods of 1-3 d, using a 7.5-m research skiff (Klimley 1993, Klimley and Holloway 1999, Klimley et al., in press). The geographic coordinates of the GS and local water temperature will be recorded at 10-sec intervals by an automated telemetry system (directional hydrophone interfaced with an ultrasonic receiver, laptop computer, and differential-corrected global positioning system to automatically

i pair the temperature measurements with geographic coordinates. GS tracks will be superimposed on bathymetric maps and satellite images of sea surface temperature using ArcView software to identify bay thermal regime preferences.

We will also track GS in the Klamath River and Yolo Bypass using radio telemetry because of anticipated poor ultrasonic signal propagation (shallow environments with large rocks as signal obstructions). Five temperature-sensing radio transmitters/year will be placed on GS in the Klamath River for intermittent (over 2 mo) tracking (car or airplane-mounted antennae) with associated environmental sampling on the river. With Yurok tribal cooperation, we will capture, tag, and track pre-spawning adult fish from the Klamath basin (D. Hillemeier, pers. com.), and data will be analyzed using at our BML laboratory to characterize the location and physical properties of their migrational/spawning habitats, essential species restoration information

We will also track five GS with radio tags in the Yolo Bypass, the primary floodplain of the Sacramento Delta. Before flowing into this basin, water must pass over the Fremont Weir, where, at all but the highest flow levels, there is an elevation difference between the Yolo Bypass and the Sacramento River at the weir. During high flow periods, upstream-migrating GS are attracted into the basin and become concentrated in a 2.4-km reach below the Fremont Weir and unable to proceed further upstream because the inadequate fish ladder at the center of the weir. We will capture and tag GS in the Yolo Bypass (in cooperation with T. Sommer, DWR), determine their residence time in the Bypass, and track them as they proceed upstream. This stranding problem is well known by CDFG wardens and recently made local TV news as a lead story. We will provide CDFG with movements (and swimming performance, see Task 1) data that may be critical in their solution of this "problem."

Task 5, Extension of CDFG Sturgeon Taming Period to Increase GS Captures: CDFG has monitored WS mortality rates and abundance since 1954 using mark-recapture techniques. Sturgeon are generally captured for tagging using trammel nets in San Pablo Bay during September and October and recaptured by anglers and during subsequent tagging operations. GS have also been captured and tagged, but in much lower numbers than WS. We suspect that higher catches of GS in September than in October is related to migratory behavior, either because of summer estuary use, as in the Columbia River estuary, or post-spawning movement out of the estuary. Because other elements of Phase 3 are dependent on CDFG GS captures (see Task 4), we propose to begin the scheduled September-October sturgeon tagging survey one month earlier in 2001 and 2002 in the expectation of capturing more GS in August than has been possible later in the year. This would make more GS available to other researchers and for movements/population estimates tagging.

Monitoring and Assessment Plans

CALFED-supported biological studies with GS are ongoing (Project No. 98-C15). For aspects of Tasks 1-3, the experimental approach, design, methods, and analyses have already been subjected to rigorous discussion and review. Detailed descriptions of all aspects of these tasks are provided in the Biological Monitoring Research and Quality Assurance Plan submitted to CALFED earlier this year and attached as Appendix 2. For Tasks 4 and 5, data collection, monitoring and assessment use standard field, laboratory, and statistical techniques (briefly described in Approach above) that will be similarly described in an updated Biological Monitoring/Research Plan. Descriptions of the current work and preliminary results have been presented at two workshops (Davis, CA, and Weitchpec, CA) and at the annual meeting of the

California-Nevada Chapter of the American Fisheries Society (Ventura, CA). In addition, a manuscript describing GS spawning, egg fertility, and larval survival has been recently submitted to the peer-reviewed *Transactions of the American Fisheries Society*.

Data Handling and Storage

Data handling and storage are described in the Biological Monitoring/Research Plan, attached as Appendix 2. These protocols will be updated as necessary for this next-phase research program.

Expected Products and Outcomes

Quarterly reports will include financial status, activities during the quarter, tasks completed, deliverables produced, problems encountered, and a description of modifications to the contract. A final technical report describing results of the studies will be submitted by the end of the project (March 31, 2003). Results of these studies have been and will continue to be presented at scientific and technical meetings (see Monitoring and Assessment Plan, above). Results of these studies will also be described in IEP Newsletter articles, and in manuscripts submitted for publication in peer-reviewed scientific journals. One manuscript from the project has been accepted for publication in the *Canadian Journal of Fisheries and Aquatic Sciences* and another submitted to the *Transactions of the American Fisheries Society*. All data will be stored by the Principal Investigator for a minimum of five years after project completion.

Work Schedule

Funding for this next-phase targeted research is requested for a two-year period beginning April 1, 2001 (expected completion date of Phase 2). The proposed work and schedule outlined below are based on seasonal sampling and year-round laboratory studies as detailed above (see Approach) and contingent on adequate funding, personnel, and fish availability. For this period, six tasks are identified (Table 1, and see Approach for specific activities involved in Tasks 1-5). Project management (Task 6) will be conducted by the Principal Investigator, J. J. Cech, Jr., assisted by the co-investigators and a research assistant.

Table 1. Tasks and schedule for proposed biological assessment of GS studies.

TASK	SCHEDULE
Task 1. GS Environmental Tolerance Limits and Behavioral Tendencies, Stress Responses and Swimming Performance	April 2001-March 2003
Task 2. Reproductive Characteristics of Wild GS, and Temperature Influences on Larval Development	April 2001-March 2003
Task 3. Genetic Analysis	April 2001-March 2003
Task 4. Telemetry	San Pablo Bay, August-September 2001-2002 Yolo Bypass, January-April 2002-2003 Klamath River, April-May 2001-2002
Task 5. Extension of CDFG Sturgeon Tagging Period to Increase GS Captures	August-September 2001-2002
Task 6. Project Management	April 2001-March 2003

Feasibility

This proposal requests next-phase funding for continuation and expansion of a successful, ongoing research program that addresses uncertainties associated with the life history of a At-Risk Priority 1 CALFED species. The project has already produced detailed quantitative data that will be used to develop GS management and conservation strategies. The targeted research outlined in this proposal is feasible, independent of the outcomes of other projects, and (generally) independent of natural conditions (e.g., weather, although inadequate supplies of wild GS could affect the rate of research). The project requires no CEQA, NEPA, or other environmental compliance documents. All necessary collecting permits (CDFG), animal care and use protocols (UC Davis), and cooperative arrangements (SFSU, Yurok Tribe) are in place. UC Davis has the appropriate laboratories and fish rearing facilities that will be required for this project. A detailed Biological Monitoring Research and Quality Assurance Plan has been approved for Phases 1 and 2. No zoning regulations, planning ordinances or other constraints that could impact the schedule and implementability of the project are known.

D. APPLICABILITY TO CALFED ERP GOALS AND IMPLEMENTATION PLAN AND CVPIA PRIORITIES (2 pages)

Relationship to ERP and CVPIA Priorities, Other Ecosystem Restoration Projects, and System-Wide Ecosystem Benefits

The GS is a CALFED at-risk species (Priority Group I, ERP Strategic Plan for Ecosystem Restoration, Table 4-1), and the proposed assessments will focus on the biological characteristics of this species and its habitats with the objective of providing information useful for their eventual recovery and protection. Our coordinated approach will resolve scientific uncertainties regarding GS life history and their spatio-temporal use of linked ecosystems (Figure 1). This will assist in GS recovery, included in CALFED Goals 1 and 3, at-risk species and harvestable species recovery and protection, as a specific ERP objective (Vol. 1, White and Green Sturgeon, pp. 146-148), and as a CVPIA goal (AFRP, pp. 40-41, 70-71, and 95). This next-phase, targeted research also contributes to the overall CALFED effort to restore ecological health and improve water management for beneficial uses of the Bay-Delta system (e.g., improved management of the Yolo Bypass for fisheries resources). This project also relates to the CALFED-funded Fish Treadmill Project (#99-N02), which aims to quantify the adverse impacts of water diversions and fish screens on GS as well as other priority species through targeted research on fish screen design and operational criteria.

Request for Next-Phase Funding

The GS project is an ongoing program, currently supported by CALFED (Project No. 98-C15, Biological Assessment of Green Sturgeon in the Sacramento-San Joaquin Watershed, J. J. Cech, Jr., Principal Investigator). This proposal requests next-phase support for Phase 3 targeted research. During the first two phases of our coordinated UC Davis-CDFG team effort, we are resolving some key areas of scientific uncertainty concerning GS' temperature-related food consumption, metabolic, growth and developmental responses; its genetic identity from white sturgeon (WS), and we are searching Feather River habitats for evidence of GS spawning. The description, current status, and scientific merit of this ongoing targeted research program are summarized in Appendix 1. We have made technical presentations of our results to date at two workshops (Davis, CA, and Weitchpec, CA) and at the annual meeting of the California-Nevada Chapter of the American Fisheries Society (Ventura, CA) in the past few months. In addition, a manuscript describing genetic variation in GS microsatellite loci has been accepted for publication by

the *Transactions of the American Fisheries Society*, and another on GS spawning, egg fertility, and larval survival has been recently submitted to the same journal. During Phase 3 (subject of this proposal), we plan to continue addressing key areas of scientific uncertainty about GS, including new studies on subadults' and adults' movements, to improve management of this species and its population(s) in the lower Sacramento-San Joaquin watershed. All of the current tasks are being continued in Phase 3, with the exception of Task 5 which will shift focus to field samples of larger GS. In addition we are adding a new task concerning telemetry of the subadult and adult GS (see Task 4 above) and two new co-investigators representing BML and SFSU.

E. QUALIFICATIONS (2 pages)

JOSEPH J. CECHE, JR., Ph.D., Professor of Fisheries Biology, UC Davis, 1987 to present.

Five Selected Publications: 1. Young, P.S. and J.J. Cech, Jr. 1996. Environmental tolerances and requirements of splittail. Trans. Am. Fish. Soc. 125:664-678. 2. Crocker, C.E. and J.J. Cech, Jr. 1997. Effects of environmental hypoxia on oxygen consumption rate and swimming activity in juvenile white sturgeon, *Acipenser transmontanus*, in relation to temperature and life intervals. Env. Biol. Fish. 50:383-389. 3. Swanson, C., P.S. Young, and J.J. Cech, Jr. 1998. Swimming performance of delta smelt: maximum performance, and behavioral and kinematic limitations on swimming at submaximal velocities. J. Exp. Biol. 201:333-345. 4. Crocker, C.E. and J.J. Cech, Jr. 1998. Effects of hypercapnia on blood-gas and acid-base status in the white sturgeon, *Acipenser transmontanus*. J. Comp. Physiol. B168:50-60. 5. Crocker, C.E., A.P. Farrell, A.K. Gamperl, J.J. Cech, Jr. 2000. Cardio-respiratory responses of white sturgeon to environmental hypercapnia. Am. J. Physiol. (in press).

SERGE I. DOROSHOV, Ph.D., Professor of Animal Science, UC Davis: 1983 to present.

Five Selected Publications: 1. Chapman, F.A., J.P. Van Eenennaam, and S.I. Doroshov. 1996. The reproductive condition of white sturgeon, *Acipenser transmontanus*, in San Francisco Bay, California. Fish. Bull. 94:628-634. 2. Van Eenennaam, J.P., S.I. Doroshov and G.P. Moberg. 1996. Spawning and reproductive performance of domestic white sturgeon (*Acipenser transmontanus*). In: S. Doroshov, F. Binkowski, T. Thuemler, D. MacKinlay (eds), Culture and Management of Sturgeon and Paddlefish (Symp. Proceedings, International Congress on the Biology of Fishes, San Francisco). pp. 117-122. 3. Van Eenennaam, J.P., S.I. Doroshov, G.P. Moberg, J.G. Watson, D.S. Moore and J. Linares. 1996. Reproductive conditions of the Atlantic sturgeon (*Acipenser oxyrinchus*) in the Hudson River. Estuaries 19(4):769-777. 4. Van Eenennaam, J.P. and S.I. Doroshov. 1999. Effects of age and body size on gonadal development of Atlantic sturgeon. J. Fish Biol. 53:624-637. 5. Webb, M.A.H., J.P. Van Eenennaam, S.I. Doroshov, and G.P. Moberg. 1999. Preliminary observations on the effects of holding temperature on reproductive performance of female white sturgeon. Aquaculture 176:315-329.

BERNARD (BERNIE) PAUL MAY, Ph.D, Adjunct Professor, 1999 to present.

Five Selected Publications: 1. Marsden, J.E., A. Spidle, and B. May. 1996. Review of genetic studies of *Dreissena* spp. Amer. Zool. 36:259-270. 2. May, B., C.C. Krueger, and H.L. Kincaid. 1997. Genetic variation at microsatellite loci in sturgeon: primer sequence homology in *Acipenser* and *Scaphirhynchus*. Can. J. Fish. Aquat. Sci. 54: 1542-1547. 3. May, B., T.A. Gavin, P.W. Sherman, and T.M. Korves. 1997. Characterization of microsatellite loci in the Northern Idaho ground squirrel, *Spermophilus brunneus* Mol. Ecol. 6:399-400. 4. May, B. 1998. Starch gel electrophoresis of allozymes. In: Molecular Genetic Analysis of Populations: A Practical Approach. 2nd Ed. A.R. Hoelzel, ed. Oxford Univ. Press. 5. McQuown, E.C., B.L. Sloss, R.J. Sheehan, J.

Rodzen, G. Tranah, and B. May. Microsatellite analysis of genetic variation in sturgeon: new primer sequences for *Scaphirynchus* and *Acipenser*. Trans. *Am. Fish. Soc.* (in press)

A. PETER KLIMLEY, Ph.D., Research Scientist, BML, Bodega Bay, 1988 to present; Lecturer, Dept. WFCB, UC Davis, 1999 to present

Five Selected Publications: 1. Klimley, A.P., B.J. Le Boeuf, K.M. Cantara, J.E. Richert, S.F. Davis, and S. Van Sommeran. 2000. Radio-acoustic positioning: a tool for studying site-specific behavior of the white shark and large marine vertebrates. *Mar. Biol.* (in press) 2. Klimley, A.P. and C. Holloway. 1999. Homing synchronicity and schooling fidelity by yellowfin tuna. *Mar. Biol.* 133: 307-317. 3. Klimley, A.P., F. Voegeli, S.C. Beavers, and B.J. Le Boeuf. 1998. Automated listening stations for tagged marine fishes. *Mar. Tech. J.*, 32: 94-101. 4. Klimley, A.P. and D.G. Ainley (Eds). 1996. *Great White Sharks: The Biology of Carcharodon carcharias*. Academic Press, San Diego. 5. Klimley, A.P. 1993. Highly directional swimming by scalloped hammerhead sharks, *Sphyrna tiburo*, and subsurface irradiance, temperature, bathymetry, and geomagnetic field. *Mar. Biol.* 117:1-22.

CARLOS E. CROCKER, Ph.D., Assistant Professor of Biology, SFSU, 2000 to present.

Five Selected Publications: 1. Deng, D.D., Refstie, S., Hemre, G.I., Crocker, C.E., Chen, H.Y., Cech, J.J., and Hung, S.S. 2000. A new technique for feeding, repeated sampling of blood and continuous collection of urine in white sturgeon. *Fish Physiol. Biochem.* (in press). 2. Crocker, C.E., Cech, J.J., Jr., Farrell, A.P., and Gamperl, K. 2000. The Effects of Hypercapnia on Cardiovascular Performance in White Sturgeon, *Acipenser transmontanus*. *Am. J. Physiol.* (in press). 3. Crocker C.E. and Cech J.J., Jr. 1998. Effects of Hypercapnia on Blood-Gas, Acid-Base Balance in White Sturgeon, *Acipenser transmontanus*. *J. Comp. Physiol. B.* 168:50-60. 4. Crocker, C.E. and Cech, J.J., Jr. 1997. The Effects of Environmental Hypoxia on Oxygen Consumption Rate and Swimming Activity in Juvenile White Sturgeon, *Acipenser transmontanus*: Temperature and Life Stage Effects. *Env. Biol. Fish.* 50:383-389. 5. Crocker, C.E. and Cech, J.J., Jr. 1996. The Effects of Hypercapnia on Growth of Juvenile White Sturgeon, *Acipenser transmontanus*. *Aquaculture.* 47: 293-299.

RAYMOND G. SCHAFFTER, M.S., Biologist, California Dept. Fish and Game 1973-present.

Five Selected Publications: 1. Schaffter, R. G. 1980. Fish occurrence, size and distribution in the Sacramento River near Hood, California during 1973 and 1974. CDFG, Anadromous Fisheries Branch Report No 80-3. 2. Schaffter, R.G., P.A. Jones, and J.G. Karlton. 1983. Sacramento River and tributaries bank protection and erosion control report. CDFG, Sacramento, CA. 3. Schaffter, R. G. 1997. White sturgeon spawning migrations and location of spawning habitat in the Sacramento River. *Calif. Fish Game* 83:1-20. 4. Schaffter, R. G. 1997. Growth of white catfish in California's Sacramento-San Joaquin Delta. *Calif. Fish Game* 84:57-67. 5. Schaffter, R.G. 1997. Mortality rates of white catfish in California's Sacramento-San Joaquin Delta. *Calif. Fish Game* 84:45-56.

DAVID W. KOHLHORST, M.A., Senior Biologist (Specialist), CDFG, 1995 to present.

Five Selected Publications: 1. Kohlhorst, D.W. 1979. Effect of first pectoral fin ray removal on survival and estimated harvest rate of white sturgeon in the Sacramento-San Joaquin Estuary. *Calif. Fish Game* 65:173-177. 2. Kohlhorst, D.W. 1980. Recent trends in the white sturgeon population in California's Sacramento-San Joaquin Estuary. *Calif. Fish and Game* 66:210-219. 3. Kohlhorst, D.W., L.W. Miller, and J.J. Orsi. 1980. Age and growth of white sturgeon collected in the Sacramento-San Joaquin Estuary, California, 1965-1970 and 1973-1976. *Calif. Fish Game* 66:83-95.

4. Kohlhorst, D.W., L.W. Botsford, J.S. Brennan, and G.M. Cailliet. 1991. Aspects of the structure and dynamics of an exploited central **California** population of white sturgeon (*Acipenser transmontanus*). Pages 277-292 ~~in~~ P. Willot, editor. *Acipenser: First International Symp. on the Sturgeon*. CEMAGREF, Bordeaux, France. 5. Stevens, D.E., D.W. Kohlhorst, L.W. Miller, and D.W. Kelley. 1985. The decline of striped bass in the Sacramento-San Joaquin Estuary, California. *Trans. Am Fish. Soc.* 114:12-30.

F. COST (3 pages excluding tables)

Budget

CALFED next-phase funding is requested for a two-year period to support continued *GS* research. Cost of the project depends on funding source: \$505,169 if funded through a State agency and \$641,362 ~~if~~ funded through a federal agency. Details of the budget, including overhead rates, are described in Tables 2 and 3 (MS Excel ~~file~~ name: *gs.calfed2000.xls*, worksheet 1=budget with state overhead rates, worksheet 2=budget with federal overhead rates) and in the Budget Justification below.

Budget Justification

Task 1: Funding is requested for support of two graduate students (75% time @ 0.0175 salary/benefits, plus student fee remissions for 2 years), supplies/rentals (fish food, reagents, chemicals, gases, molecular biology supplies, steroid analyses supplies, and physiological measurements supplies, assays, office supplies, tank rental charges), travel for specimen collection and meeting/workshop attendance, and University of **California, Davis**, overhead.

Task 2: Funding is requested for one research associate (50% time @ 0.24 salary/benefits for 5 months/year for 2 years), one laboratory assistant (100% time @ 0.24 salary/benefits for 2 years), supplies/rentals (fish food, reagents, chemicals, histological supplies, film and developing, ~~assays~~, office supplies, tank rental charges), travel for specimen collection and meeting/workshop attendance, and University of California, Davis, overhead.

Task 3: Funding is requested for one adjunct professor (17% time @ 0.25 salary/benefits for 2 years), one technician (33% time @ 0.25 ~~salary/benefits~~ for 2 years), supplies/rentals (reagents, chemicals, gases, office supplies), equipment (\$4000 for two gel rigs and one power supply), travel for specimen collection and meeting/workshop attendance, and University of California, Davis, overhead.

Task 4a: Funding is requested for one research scientist (25% time @ 0.25 salary/benefits for 2 years), one graduate student (75% time @ 0.0175 salary/benefits, plus student fee remission for 2 years), supplied rentals (boat fuel, transmitters, airplane rental, office supplies), equipment (\$5060 for ultrasonic and radio receivers), travel for specimen tracking and meeting/workshop attendance, and Bodega Marine Laboratory overhead.

Task 4 b Funding ~~is~~ requested for support of one graduate student (50% time @ **0.1** salary/benefits for 6 months for each of the 2 years) and San Francisco State University overhead.

Task 5: Funding is requested for one CDFG boat operator (100% time @ 0.32 salary/benefits for 3 months/year for 2 years), supplied rentals (nets and net repair supplies, gases and chemicals, office

Table 2. Budget for two years of project with 10% (state) overhead rate.

Year	Task	Direct Labor Hours	Subject to Overhead						Exempt from Overhead		Total Cost
			Salary	Benefits	Travel	Supplies & Expendables	Subcontractor Overhead (@ 51% for Task 4b and Task 5) @20% of all tasks	Overhead (10% for all tasks)	Equipment	Graduate Student Fee	
Year 1	Task 1	3000	\$40,000	\$700	\$2,000	\$9,000	\$0	\$5,170	\$0	\$9,000	\$65,870
	Task 2	1250	\$23,625	\$5,670	\$1,000	\$5,000	\$0	\$3,530	\$0	\$0	\$38,825
	Task 3	999	\$24,600	\$6,150	\$600	\$6,000	\$0	\$3,735	\$4,000	\$0	\$45,085
	Task 4a	2000	\$35,802	\$4,384	\$800	\$11,199	\$0	\$5,219	\$5,060	\$4,756	\$67,220
	Task 4b	500	\$7,500	\$825	\$0	\$0	\$4,246	\$1,257	\$0	\$0	\$13,828
	Task 5	848	\$12,335	\$2,467	\$0	\$2,910	\$3,525	\$2,124	\$0	\$0	\$23,361
	Task 6	50	\$500	\$9	\$0	\$250	\$0	\$76	\$0	\$0	\$835
Total Cost Year 1			\$144,362	\$20,205	\$4,400	\$34,359	\$7,771	\$21,111	\$9,060	\$13,756	\$255,024
Year 2	Task 1	3000	\$40,000	\$700	\$2,000	\$9,000	\$0	\$5,170	\$0	\$9,000	\$65,870
	Task 2	1250	\$24,334	\$5,840	\$1,000	\$5,000	\$0	\$3,617	\$0	\$0	\$39,791
	Task 3	999	\$25,830	\$6,458	\$600	\$6,000	\$0	\$3,889	\$0	\$0	\$42,777
	Task 4a	2000	\$36,519	\$4,836	\$800	\$11,199	\$0	\$5,335	\$0	\$4,994	\$63,683
	Task 4b	500	\$7,500	\$825	\$0	\$0	\$4,246	\$1,257	\$0	\$0	\$13,828
	Task 5	848	\$12,335	\$2,467	\$0	\$2,910	\$3,525	\$2,124	\$0	\$0	\$23,361
	Task 6	50	\$500	\$9	\$0	\$250	\$0	\$76	\$0	\$0	\$835
Total Cost Year 2			\$147,018	\$21,135	\$4,400	\$34,359	\$7,771	\$21,468	\$0	\$13,994	\$250,145
Total Project Cost			\$291,380	\$41,340	\$8,800	\$68,718	\$15,542	\$42,579	\$9,060	\$27,750	\$505,169

Table 3. Budget for two years of project **with** federal overhead rates (46.5-48% for tasks 1, 2, 3, 5, and 6, 26% for Task 4a).

Year	Task	Direct Labor Hours	Subject to Overhead						Exempt from Overhead		Total Cost
			Salary	Benefits	Travel	Supplies & Expendables	Subcontractor and Overhead @48% for Year 2; Task 4b and Task 5) @20% for both years)	Overhead (Tasks 1, 2, 3, 5, & 6 @46.5% for Year 1 and Overhead @48% for Year 2; Task 4b and Task 5) @26% for both years)	Equipment	Graduate Student Fee Remission	
Year 1	Task 1	3000	\$40,000	\$700	\$2,000	\$9,000	\$0	\$24,040	\$0	\$9,000	\$84,740
	Task 2	1250	\$23,625	\$5,670	\$1,000	\$5,000	\$0	\$16,412	\$0	\$0	\$51,707
	Task 3	999	\$24,600	\$6,150	\$600	\$6,000	\$0	\$17,368	\$4,000	\$0	\$58,718
	Task 4a	2000	\$35,802	\$4,384	\$800	\$11,199	\$0	\$13,568	\$5,060	\$4,756	\$75,569
	Task 4b	500	\$7,500	\$825	\$0	\$0	\$4,246	\$5,846	\$0	\$0	\$18,417
	Task 5	848	\$12,335	\$2,467	\$0	\$2,910	\$3,525	\$9,875	\$0	\$0	\$31,115
	Task 6	50	\$500	\$9	\$0	\$250	\$0	\$353	\$0	\$0	\$1,112
Total Cost Year 1			\$144,362	\$20,205	\$4,400	\$34,359	\$7,771	\$87,462	\$9,060	\$13,756	\$632,375
Year 2	Task 1	3000	\$40,000	\$700	\$2,000	\$9,000	\$0	\$24,816	\$0	\$9,000	\$85,516
	Task 2	1250	\$24,334	\$5,840	\$1,000	\$5,000	\$0	\$17,364	\$0	\$0	\$53,538
	Task 3	999	\$25,830	\$6,458	\$600	\$6,000	\$0	\$18,666	\$0	\$0	\$57,554
	Task 4a	2000	\$36,519	\$4,836	\$800	\$11,199	\$0	\$13,872	\$0	\$4,994	\$72,220
	Task 4b	500	\$7,500	\$825	\$0	\$0	\$4,246	\$6,034	\$0	\$0	\$18,605
	Task 5	848	\$12,335	\$2,467	\$0	\$2,910	\$3,525	\$10,194	\$0	\$0	\$31,431
	Task 6	50	\$500	\$9	\$0	\$250	\$0	\$364	\$0	\$0	\$1,123
Total Cost Year 2			\$147,018	\$21,135	\$4,400	\$34,359	\$7,771	\$91,310	\$0	\$13,994	\$319,987
Total Project Cost			\$291,380	\$41,340	\$8,800	\$68,718	\$15,542	\$178,772	\$9,060	\$27,750	\$641,362

supplies), and CDFG and University of California, Davis, overhead.

Task 6: Funding is requested for support of research assistant (2.5% time @ 0.0175 salary/benefits for 2 years) and office supplies.

Cost Sharing

“Leveraged” support (\$85,478) will be provided by UC Davis (5% of two investigators’ salaries and benefits while working on the GS project), and an estimated \$60,000 of support by using CDFG personnel and vessels conducting white sturgeon (WS) research funded by Federal Sport Fisheries Restoration Funds and matching state funds for obtaining GS, and by using State Water Project facilities and personnel to collect juveniles at the Byron fish screens.

G. LOCAL INVOLVEMENT

Most of the infrastructure/equipment required for this project is already available at UC Davis, Bodega Marine Laboratory, SFSU Romberg Center for Marine Studies, and CDFG Bay Delta and Special Water Projects Division. Collaboration with the Yurok and Karuk Tribal Fisheries biologists has been arranged or initiated. Increased knowledge of this priority (at-risk) species will potentially assist several CALFED projects.

H. COMPLIANCE WITH STANDARD TERMS AND CONDITIONS

The University of California, Davis, and the California Department of Fish and Game are public organizations of the State of California. Both organizations comply with the standard terms and conditions of non-discrimination and non-collusion. There are no conflicts of interest.

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J. THRESHOLD REQUIREMENTS

UC Davis, Bodega Marine Laboratory, and San Francisco State University are State-assisted public research and educational institutions. California Department of Fish and Game is a Constitutionally mandated agency of the State of California. (Non-Profit, exempt under status 501(c)(3) of the IRS code of 1954 under Type of organization and Tax Status). Tax Identification Number for UC Davis is 94-603-6494. Also see attached documents.

Appendix I

CURRENT PROJECT STATUS SUMMARY (CALFED #98-C15)

This project is a cooperative, targeted research program that will provide valuable information to decision-makers using adaptive management to resolve scientific uncertainties in our *GS* life history conceptual model (Figure 1) and assist in *GS* recovery, a specific CALFED and CVPLA goal. Current status of the five current tasks of Phase 1 (Phase 2 just starting) is described below.

Task 1: *GS* Bioenergetics (Phase I activities, 95% complete)

Young-of-the-year (YOY) green sturgeon (*Acipenser medirostris*) (*GS*), spawned from Klamath River-collected broodstock *GS* in May, 1999, in cooperation with the Yurok Tribe (see Tasks 2, 3, 4), were used in a series of respiratory metabolism, food consumption, growth and temperature preference experiments. *GS* routine metabolic rates were measured at 11, 19, and 24°C, with rates generally increasing with increasing temperature and increasing body weight. Other YOY *GS* were situated in replicate rearing tanks at three temperatures: (11, 15, and 19°C) and two ration levels (ad lib. and 50% ad lib.), and food consumption and growth rates were measured over a 30-day period. Increases in temperature and ration size generally increased juvenile *GS* food consumption and growth rates. Food conversion efficiency was higher at the reduced ration and at the warmer (19 and 24°C) temperatures compared with the ad lib. (satiation) ration and cooler (11°C) temperature, respectively. Summaries of the results collected to date were presented at the *GS* Workshop (Weitchpec, CA, 3-22-00) and at the annual meeting of the California-Nevada Chapter of the American Fisheries Society (Ventura, CA, 3-31-00).

Task 2: Reproductive Characteristics of Wild *GS* (Phase 1 activities, 95% complete)

Body size data, samples of gonads and fin rays have been collected from 14 female and 24 male adult green sturgeon (*GS*) by our Yurok tribe collaborators on the Klamath River. Histological processing of gonad samples and preparation of fin ray sections for aging have been completed and currently the descriptions and microphotography of the histological sections and scoring the fin ray sections for age are ninety-percent completed.

GS embryos and larvae from the Klamath River-collected broodstock spawning were sampled through metamorphosis for body size measurements, morphometric analyses, and photography. Fertilized eggs hatched after incubating 7 days at 15.3°C. Hatched larvae were 13.8 mm total length had large ovoid yolk sacs and were strongly photonegative. Unlike other sturgeons, *GS* larvae did not exhibit a vertical **swim-up** behavior upon hatching. Rather, they aggregated in clumps at the bottom of the tank or swam along the outside edge of the tanks, *against* the water current. They are less active during the day and spend most of the time at the bottom. During night, they swim vigorously along the walls of the tank. Exogenous feeding begins at ca. 12-13 days posthatch at 18.5°C (mean water temperature). At 9 months posthatch the *GS* weighed 1,012 grams (mean wet weight), indicating a much faster growth rate than similar-age white sturgeon (*Acipenser transmontanus*), which weighed 500 grams at UCD and the nearby commercial farms. A manuscript describing the *GS* spawning, egg fertility and larval survival has been submitted to the *Transactions of the American Fisheries Society*.

Task 3: Assessment of Stress (Phase 1 activities, 70% complete)

Development of the ability to respond to stressful events with the synthesis and release of corticosteroids (hormones that **are** associated with the general stress response in most vertebrates) **has** proven to have an irregular onset across vertebrate species. Knowledge gained from understanding when *GS* develop the capability to mount a stress response could be utilized to improve spawning and rearing techniques along with identifying the best time, in terms of stress, to

transport animals. Beginning 8 days post hatch (dph) we measured the stress response, in terms of whole body corticosteroids, of young-of-the-year (YOY) GS and white sturgeon (WS) larvae to a 30-second air emersion. The corticosteroids were measured by radioimmunoassay techniques employed on whole body homogenates. Our results suggest that the green sturgeon has the ability to synthesize corticosteroids as early as 8 dph, the earliest reported maturation of the stress axis for all fishes. In contrast, WS larvae did not show a significant change in corticosteroids concentration until 15 dph, which is similar to the timing reported for most "modem" (teleostean) bony fishes. In addition, we recently investigated differences in the GS's diurnal and nocturnal stress responses. We exposed groups of 6-month-old YOY to 1-minute air emersions and collected blood and liver samples at predetermined intervals during their recovery from this standardized stress. Plasma cortisol, glucose, and lactate levels and liver glycogen levels (important indicators of physiologic stress) are all being measured in our laboratories. Besides physiologically defining the GS stress response for the first time, this study (with appropriate statistical comparisons) will quantify day and night differences. Finally, 16 individual YOY GS were chronically cannulated for repeated blood sampling with minimal sampling-related stress, held in separate tanks, and sampled before and after air emersion to detect temperature-related stress response differences. GS plasma cortisol, glucose, and lactate levels will be measured and compared using appropriate statistical models.

Task 4: Genetic Analysis (Phase 1 activities, 90% complete)

This task has two objectives during phase 1 of this project, (1) to develop species-specific genetic markers for green sturgeon (GS) and white sturgeon (WS) and (2) to develop intraspecific nuclear genetic markers that could be used in a phase 2 study to differentiate GS populations. The first objective uses two approaches. A mitochondrial (mt) DNA marker was developed that uses an Ssp1 restriction (enzyme) site presence in cytochrome B in GS that is absent in WS. Amplification and subsequent digestion with Ssp1 yields a single sequence in WS and two smaller sequences in GS. Secondly, amplified fragment length polymorphisms (AFLPs) were examined in GS and WS that showed numerous fixed differences between these species. Several of these bands were cut out of gels and sequenced. Primers were developed for one of these differences that shows a seven-base pair deletion in GS versus WS DNA to determine the identity of any size sturgeon, including fry. We are continuing to develop additional interspecific markers. Insufficient intraspecific differences were seen in AFLPs in GS to justify pursuing our second objective with AFLPs. Therefore, we have concentrated on the development of highly polymorphic microsatellite markers for GS. We have been redesigning and testing primers we developed for other sturgeon species to work in GS. We have about six loci that should prove useful for population differentiation analysis in phase 2, and we have been testing them on larger numbers of samples. The polyploid (octoploid) derivative nature of this organism makes it very difficult to develop usable nuclear markers.

Task 5: Determination of GS Spawning Habitats and Environmental Conditions (phase 1 activities, 30% complete)

Field sampling began in February, 2000, and artificial substrates have been set at 6 locations in the Feather River between the Thermalito Afterbay outlet (Lat. 39° 27.23', Long. 121° 38.35') and Shanghai Bend (Lat. 39° 5.41', Long. 121° 35.93') generally downstream to upstream migrational impediments. Twice-weekly retrieval of artificial substrates have yielded no sturgeon eggs, to date. Locations for larval net sampling have been established at 3 locations and preliminary daytime and nighttime sampling has begun. Preliminary daylight larval net sampling has yielded only larval Sacramento suckers (*Catostomus occidentalis*).

APPENDIX 2

BIOLOGICAL ASSESSMENT OF GREEN STURGEON IN THE SACRAMENTO-SAN JOAQUIN WATERSHED

BIOLOGICAL MONITORING/RESEARCH & QUALITY ASSURANCE PLAN

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Biological Monitoring/Research and Quality Assurance Approach and Methods (Phase 1):

Animal Sampling and Holding: Juvenile and subadult/adult GS will be collected primarily from the Feather and Sacramento Rivers using appropriate methods and gear (specific methods outlined in Task 5, below) and held at UC Davis. Complete catch records (both electronic and hard copies) will be maintained by CDFG. Fish will be transported to the UC Davis Aquatic Center in oxygenated plastic bags (juveniles) or tanks (subadults/adults) with river water that will be kept cool. Immediately upon arrival, fish will be transferred to 1-4-m diameter, fiberglass tanks with aeration and a continuous flow of unchlorinated, air-equilibrated well water. Tank temperatures will approximate river temperatures at the time of capture (except for specific experimental protocols, see below), and GS will be offered fish pellets *ad libitum* (except for specific experimental protocols, see below).

Phase 1 Tasks: Task 1. **GS Temperature Tolerance Limits and Behavioral Tendencies and Swimming Performance:** Temperature and current play important roles in the development and survival of young fish. Determination of **GS** acute temperature tolerance **limits** (critical thermal **maxima** and **minima**) will follow modifications of the Becker and Genoway (1979) method (10 replicates, 1°C increasing or decreasing temperature per 10 min), using the loss of equilibrium endpoint. Control fish will be subjected to the same protocol but without temperature changes. At the endpoint, two stopcocks will be immediately switched, flushing the vessel with ambient water to quickly recover the fish. Loss of equilibrium in fish indicates physical disorganization due to the experimental variable and loss of the fish's ability to escape from conditions leading to its death (Becker and Genoway 1979). Horizontal, temperature gradient tanks (1.5 m long) will be used for **GS'** (acclimated to 11, 15, or 19°C) behavioral tendencies (temperature selection) experiments. Water depth will be set to avoid vertical temperature stratification of the water, yet avoid alarming the test fish. Fresh water (10°C and 25°C) will continuously flow into opposite ends of the gradient tank, and a telethermometer/probes system and angled mirrors will allow observations (every 5 min during the 1-h experimental period) of individual **GS'** temperature selections without disturbance. Controls will use ambient temperature water flows from both ends of the tank. **GS** critical swimming velocities (U_{crit}) will be determined at 11, 15, and 19°C using a modified Brett-type recirculating water flume incorporating a variable-speed motor (Brett 1964). Juvenile fish will be placed in the swimming chamber of the 9-l flume and, after a 60-min acclimation period, critical swimming velocity will be measured by step increases of 10 cm/s in water velocity at 10 min intervals starting at 10 cm/s until the fish is fatigued (Beamish 1978, Young and Cech 1996). Appropriate statistical models (ANOVA, Kruskal-Wallis, and post-hoc tests) will be used to compare **GS** size and acclimation temperature group means, and duplicate (electronic and hard-copy) data records will be kept for all experiments.

: Sturgeon have low reproductive rates because of their late sexual maturity and long intervals between the consecutive spawnings. Reproductive characteristics and reproductive rates vary in different species and populations, and may be determining factors in their resilience to extinction under continuing fishery and partial loss of the spawning habitat. It is important to obtain baseline information (not yet known) on wild **GS** reproductive development and traits, as in our studies with Atlantic and white (WS) sturgeons (Van Eenennaam et al. 1996, Doroshov et al. 1997, Chapman et al. 1996). We will collect samples of gonads and pectoral finrays (along with body size and capture site data) from juvenile, subadult, and adult **GS** (30-60 fish) captured in the Sacramento River system, Klamath River, and in their estuaries. Gonadal tissue will be histologically processed and the slides examined to identify sex, stage of gametogenesis, and morphological characteristics of gonadal tissue and germ cells. Ovaries will be subsampled for the individual fecundity, oocyte size, and germinal vesicle migration (criterion of oocyte ripeness and closeness of female to spawning; Van Eenennaam et al. 1996, Doroshov et al. 1997). Age will be reconstructed from the analysis of finray sections. Appropriate statistical models (ANOVA, Kruskal-Wallis, and post-hoc tests) will be used to compare **GS** sample means, and duplicate (electronic and hard-copy) data records will be kept for all groups.

Task 3. Assessment of Stress and its Impact on Reproduction: Stress negatively impacts the health, growth and reproductive success of fish. We **will** first characterize the quantitative and qualitative nature of the GS' stress response by defining their interrenal response and correlate this response to changes in reproductive hormones. GS **will** be maintained at the UC Davis Aquatic Center in outdoor tanks with continuous running water under natural light, permitting water quality and temperature monitoring, and continual supervision of trained staff. Prior to experimentation, GS gonadal tissue **will** be removed to determine sex and stage of reproductive development of each fish (see Task 2, above). To characterize the interrenal response of these fish, individual fish **will** be anesthetized with MS 222, and fitted with an indwelling cannula (caudal vein) for subsequent blood sampling. Blood samples **will** be analyzed for cortisol, testosterone, 17α , 20β DH-pregnenolone and estradiol using radioimmunoassay (Moberg et al., 1995; Faulkner and Moberg, 1997). We **will** also determine circadian cortisol secretion rhythms in both males and females. Appropriate statistical models (ANOVA, Kruskal-Wallis, and post-hoc tests) **will** be used to compare GS group means, and duplicate (electronic and hard-copy) data records **will** be kept for all experiments.

Task 4. Genetic Analysis: We **will** use two molecular approaches (microsatellites and amplified fragment length polymorphisms, AFLPs) to address questions regarding the GS' genetic health (genetic variability) and the genetic integrity (population structure) in the Sacramento-San Joaquin basin. We **will** assess GS variability and develop species-specific markers to distinguish between GS and WS and to screen collected embryos. Recently, we found six of the eleven microsatellite loci to amplify well in GS (May et al. 1997). A very new technique (amplified fragment length polymorphisms, AFLPs) for rapidly screening large portions of the genome has recently become available (Vos et al. 1995), and we have successfully used AFLPs to identify subspecies of endangered tui chubs (*Gila bicolor*), to build a linkage map for tilapia (*Oreochromis* and *Seraphodon*), and to identify differences among populations of *Myxobolus cerebralis*, the parasite which causes whirling disease in fish. This approach leads to a variable number of bands per individual (with a target of 10-50 bands) depending primarily on the size of the genome and the number of base extensions used per primer. Variation is usually scored as presence or absence of bands; however, in many cases variation can be noted as sequence length differences. Whole embryos or fin samples **will** be collected and stored in 95% ethanol. Fin sections are taken with utility razor blades and these are exchanged and dissection boards rinsed with water and 70% ethanol between individuals. Genomic DNA **will** be extracted using the CTAB phenol/chloroform protocol (Saghai-Marooft et al. 1984, Doyle and Doyle 1987, Grewe et al., 1993). Appropriate statistical models (ANOVA, Kruskal-Wallis, and post-hoc tests) **will** be used to compare GS sample group means, and duplicate (electronic and hard-copy) records **will** be kept for all data sets.

Task 5. Determination of Sturgeon Spawning Habitats and Their Environmental Conditions: Insulated aeration chambers **will** be constructed for transport of eggs and larvae collected from the Feather River. Artificial substrate **will** be fished continuously from March through June at 6 sites, between Shanghai Bend (Rkm 41) and Thermalito outfall (Rkm 95) which have depth, velocity and substrate characteristics typical of spawning sites of other sturgeon. Substrates **will** be retrieved

twice weekly, all eggs will be either preserved in fixatives compatible with later DNA analysis (microsatellite and AFLPs) to determine species (WS or GS) or will be transported alive to UC Davis for growout for species determination. Preserved embryo samples will be aged to back-calculate time of spawning using temperature-modified WS development times (Wang et al. 1985, Beer 1981) until species-specific information is developed from our captive breeding and culture studies. Twice weekly, larval nets (Kohlhorst 1976) will be fished at locations between the southern end of the Oroville Wildlife area (Rkm 87) and the Highway 99 bridge near Nicklaus (Rkm 15). Larvae will be either preserved or maintained alive for transport to UC Davis. Time of spawning will be estimated by WS larval development times (Beer 1981). Throughout the spawning seasons, flow will be monitored hourly by the CDWR at established flow recording stations immediately below the Thermalito outfall (Rkm 95), near the Gridley bridge (Rkm 81), near Yuba City (Rkm 45) and in the Yuba River near Marysville. This study will establish hourly temperature recording stations above the Thermalito outfall (Rkm 96), Gridley (Rkm 91), below the Yuba River, and near Nicklaus (Rkm 15) using submersible data loggers that will be weekly interrogated during late winter/spring. During deployment of artificial substrates, water velocity 30 cm above the substrate will be measured (current meter), bottom (substrate) samples will be taken with an Ekman dredge, and substrates will be indexed (Instream Flow Suitability Method). In San Pablo Bay and the west Delta, CDFG sturgeon gill-netting procedures will be modified and a live tank constructed to maintain captured GS alive for transport to UC Davis. We will also facilitate/coordinate the storage of GS juveniles captured at the John Skinner Fish Facility of the State Water Project for later transport to UC Davis. Appropriate statistical models (ANOVA, Kruskal-Wallis, and post-hoc tests) will be used to compare GS sample group means, and duplicate (electronic and hard-copy) records will be kept for all data sets.

Approach and Methods, (Phase 2): GS will be sampled and held as in Phase 1 (above). **Task 1, Food Consumption Rate, Growth Rate, and Respiratory Metabolism Measurements.** Juvenile GS, either from river and Delta collections or from captive breeding experiments (see below) will be used to assess temperature's effects on three critical functions: food consumption, growth, and metabolism. Food consumption rate and growth rate studies will be conducted simultaneously on juvenile GS in replicate tanks at three temperature treatments: 11, 15, and 19°C (Myrick and Cech 1996). Fish will be situated in groups of 30 in five, replicate 110-L round fiberglass tanks (with continuous water and air flows) per temperature treatment. Fish will be fed Biodiet fish pellets twice daily, and uneaten pellets will be siphoned (and counted) twice daily to calculate food consumption rates (in g food/tank/day, with appropriate statistical comparisons between groups). Fish will be weighed and measured at the start and end of the 30-d experiment to determine growth rates, using appropriate statistical comparisons. Specific growth rate will be determined (Busacker et al. 1990) for comparisons with literature values on juvenile WS (Cech et al. 1984, Crocker and Cech 1996). Respiratory metabolic (oxygen consumption) rate measurements will be conducted on ten (post-feeding consumption and growth rates experiment) fish from each of the three temperature treatment groups (11, 15, 19°C) in closed respirometers following the methods of Cech (1990). If GS show significant activity in respirometers, experiments will be videotaped to quantify activity (Crocker and Cech 1997) and oxygen (convertible to energy units) costs of activity will be estimated (with appropriate statistics).

Appropriate statistical models (ANOVA, Kruskal-Wallis, and post-hoc tests) **will** be used to compare GS size and acclimation temperature group means, and duplicate (electronic and hard-copy) data records **will** be kept for all experiments.

Task 2. Captive Breeding, Culture, and Characterization of Early Developmental Stages; GS captive breeding **will** provide critical material for our assessments, in addition to experience and techniques that may be needed for artificial reproduction of this rare species. We **will** collect 2-3 female and 3-5 male broodfish from the Sacramento and Klamath systems (gill nets or angling) and transport them by truck (with special oxygenated tank) to UC Davis. Broodfish **will** be held in 4-m diameter outdoor tanks with **continuous** water flows. Spawning and hatchery techniques will generally follow standard WS procedures (Conte et al. 1988, Van Eenennaam et al. 1996, Moberg and Doroshov 1996). Larvae and juveniles **will** be raised, at low density in smaller flow-through tanks, on the **artificial** (Biodiet) and/or natural (brine shrimp nauplii and tubifex) diets. Young GS survival, growth, feeding, and health **will** be maintained, and water **quality will** be monitored. We expect success, because Asian GS have large yolky eggs, and large and robust larvae at the **onset of** exogenous feeding (Artyukhin and Andronov, 1990), in contrast with lake and Atlantic sturgeon that possess more technically challenging larvae. We will incubate fertilized GS eggs (petri dishes or glass trays) in temperature-controlled (four temperatures: range 8- 18°C) flow-through tanks (or in hatching jars at 10-20°C) and monitor development rates (including mortalities and abnormalities) using photomicrography and regression analysis (Wang et al. 1985, Dettlaff et al. 1993). Larval measurements and weights **will** yield temperature effects data **on larval growth**. Photomicrographs of embryos and larvae **will** be scanned, processed (Adobe Photoshop software) and compared with WS. Dettlaff et al. (1993) noted that species-specific differences in sturgeon are usually subtle during the embryo development, and different species are usually distinguished by the egg size and pigmentation patterns; however, the differences in morphology become prominent in larval stages, particularly before the transition to exogenous feeding (Wang et al. 1985). Appropriate statistical models (ANOVA, Kruskal-Wallis, and post-hoc tests) will be used to compare GS sample means, and duplicate (electronic and hard-copy) data records **will** be kept for all groups.

Task 3. Responses to Stressors; GS' responses to stressors will be determined via brief **air** exposures in a dip net (simulating culture procedures) and ACTH₁₋₂₄ administrations (via the vascular cannula) to determine the **maximum** and temporal characteristics of the GS' interrenal response. At this time and during subsequent studies, we **will** monitor the effect of the interrenal response on gonadal steroid secretion. These data **will** be used to establish appropriate culture conditions (e.g., tank size, stocking densities, handling practices, water temperature), should GS captive breeding/mitigative stocking be needed. Appropriate statistical models (ANOVA, Kruskal-Wallis, and post-hoc tests) **will** be used to compare GS group means, and duplicate (electronic and hard-copy) data records **will** be kept for all experiments.

Task 4. Delta GS Stock Identification: The degree of reproductive isolation of Sacramento-San Joaquin GS from Klamath River and Rogue River (Oregon) populations will be determined using the microsatellites and AFLP DNA techniques employed in Phase I studies. Klamath River

collections (coordinated with Troy Fletcher [Yurok Tribe]) and Rogue River (coordinated with ODFW biologists) will quantitatively describe the regional uniqueness of Sacramento-San Joaquin GS. Appropriate statistical models (ANOVA, Kruskal-Wallis, and post-hoc tests) will be used to compare GS sample group means, and duplicate (electronic and hard-copy) records will be kept for all data sets.

Task 5. Determination of Sturgeon Spawning Habitats and Their Environmental Conditions: GS sampling and spawning habitat characterization will continue using the equipment and techniques that proved most successful during Phase 1 efforts. Appropriate statistical models (ANOVA, Kruskal-Wallis, and post-hoc tests) will be used to compare GS sample group means, and duplicate (electronic and hard-copy) records will be kept for all data sets.

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NONDISCRIMINATION COMPLIANCE STATEMENT

STD. 19 (REV. 3-95)

COMPANY NAME

The company named above (hereinafter referred to as "prospective contractor") hereby certifies, **unless** specifically exempted, compliance with Government Code Section **12990** (a-f) and California Code of Regulations, Title 2, Division **4**, Chapter 5 in matters relating to reporting requirements and the development, implementation and maintenance of a Nondiscrimination Program. Prospective contractor agrees not to unlawfully discriminate, harass or allow harassment ~~against any~~ employee or applicant for employment because of sex, ~~race~~, color, ancestry, religious ~~creed~~, national origin, physical disability (including HIV and AIDS), medical ~~condition~~ (cancer), age (over 40), marital status, denial of family care leave and denial ~~of~~ pregnancy disability leave.

CERTIFICATION

I, the official named below, hereby swear that I am duly authorized to legally bind the prospective contractor to the above described certification. I am fully aware that this certification, executed on the date and in the county below, is made under penalty of perjury under the laws of the State of California.

:

OFFICIAL'S NAME

THE REGENTS OF THE UNIVERSITY
OF CALIFORNIA

DATE EXECUTED

MAY 12 2008

EXECUTED IN THE COUNTY OF

Yolo

PROSPECTNECOMRACTOR'S SIGNATURE



PROSPECTIVE CONTRACTOR'S TITLE

Sandra M. Dowdy
Contracts and Grants Analyst

PROSPECTIVE CONTRACTOR'S LEGAL BUSINESS NAME

ASSURANCES - NON-CONSTRUCTION PROGRAMS

Public reporting burden for this collection of information is estimated to average 15 minutes per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding the burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to the Office of Management and Budget, Paperwork Reduction Project (0348-0040), Washington, DC 20503.

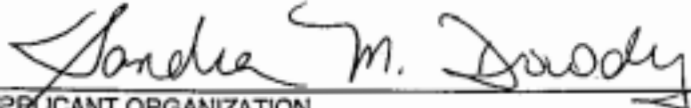
PLEASE DO NOT RETURN YOUR COMPLETED FORM TO THE OFFICE OF MANAGEMENT AND BUDGET. SEND IT TO THE ADDRESS PROVIDED BY THE SPONSORING AGENCY.

NOTE: Certain of these assurances may not be applicable to your project or program. If you have questions, please contact the awarding agency. Further, certain Federal awarding agencies may require applicants to certify to additional assurances. If such is the case, you will be notified.

As the duly authorized representative of the applicant, I certify that the applicant:

1. Has the legal authority to apply for Federal assistance and the institutional, managerial and financial capability (including funds sufficient to pay the non-Federal share of project cost) to ensure proper planning, management and completion of the project described in this application.
2. Will give the awarding agency, the Comptroller General of the United States and, if appropriate, the State, through any authorized representative, access to and the right to examine all records, books, papers, or documents related to the award; and will establish a proper accounting system in accordance with generally accepted accounting standards or agency directives.
3. Will establish safeguards to prohibit employees from using their positions for a purpose that constitutes or presents the appearance of personal or organizational conflict of interest, or personal gain.
4. Will initiate and complete the work within the applicable time frame after receipt of approval of the awarding agency.
5. Will comply with the Intergovernmental Personnel Act of 1970 (42 U.S.C. §§4728-4763) relating to prescribed standards for merit systems for programs funded under one of the 19 statutes or regulations specified in Appendix A of OPM's Standards for a Merit System of Personnel Administration (5 C.F.R. 900, Subpart F).
6. Will comply with all Federal statutes relating to nondiscrimination. These include but are not limited to: (a) Title VI of the Civil Rights Act of 1964 (P.L. 88-352) which prohibits discrimination on the basis of race, color or national origin; (b) Title IX of the Education Amendments of 1972, as amended (20 U.S.C. §§1681-1683, and 1685-1686), which prohibits discrimination on the basis of sex; (c) Section 504 of the Rehabilitation Act of 1973, as amended (29 U.S.C. §794), which prohibits discrimination on the basis of handicaps; (d) the Age Discrimination Act of 1975, as amended (42 U.S.C. §§6101-6107), which prohibits discrimination on the basis of age; (e) the Drug Abuse Office and Treatment Act of 1972 (P.L. 92-255), as amended, relating to nondiscrimination on the basis of drug abuse; (f) the Comprehensive Alcohol Abuse and Alcoholism Prevention, Treatment and Rehabilitation Act of 1970 (P.L. 91-616), as amended, relating to nondiscrimination on the basis of alcohol abuse or alcoholism; (g) §§523 and 527 of the Public Health Service Act of 1912 (42 U.S.C. §§290 dd-3 and 290 ee 3), as amended, relating to confidentiality of alcohol and drug abuse patient records; (h) Title VIII of the Civil Rights Act of 1968 (42 U.S.C. §§8601 et seq.), as amended, relating to nondiscrimination in the sale, rental or financing of housing; (i) any other nondiscrimination provisions in the specific statute(s) under which application for Federal assistance is being made; and, (j) the requirements of any other nondiscrimination statute(s) which may apply to the application.
7. Will comply, or has already complied, with the requirements of Titles II and III of the Uniform Relocation Assistance and Real Property Acquisition Policies Act of 1970 (P.L. 91-646) which provide for fair and equitable treatment of persons displaced or whose property is acquired as a result of Federal or federally-assisted programs. These requirements apply to all interests in real property acquired for project purposes regardless of Federal participation in purchases.
8. Will comply, as applicable, with provisions of the Hatch Act (5 U.S.C. §§1501-1508 and 7324-7328) which limit the political activities of employees whose principal employment activities are funded in whole or in part with Federal funds.

9. Will comply, as applicable, with the provisions of the Davis-Bacon Act (40 U.S.C. §§276a to 276a-7), the Copeland Act (40 U.S.C. §276c and 18 U.S.C. §874), and the Contract Work Hours and Safety Standards Act (40 U.S.C. §§327-333), regarding labor standards for federally-assisted construction subagreements.
10. Will comply, if applicable, with flood insurance purchase requirements of Section 102(a) of the Flood Disaster Protection Act of 1973 (P.L. 93-234) which requires recipients in a special flood hazard area to participate in the program and to purchase flood insurance if the total cost of insurable construction and acquisition is \$10,000 or more.
11. Will comply with environmental standards which may be prescribed pursuant to the following: (a) institution of environmental quality control measures under the National Environmental Policy Act of 1969 (P.L. 91-190) and Executive Order (EO) 11514; (b) notification of violating facilities pursuant to EO 11738; (c) protection of wetlands pursuant to EO 11990; (d) evaluation of flood hazards in floodplains in accordance with EO 11988; (e) assurance of project consistency with the approved State management program developed under the Coastal Zone Management Act of 1972 (16 U.S.C. §§1451 et seq.); (f) conformity of Federal actions to State (Clean Air) Implementation Plans under Section 176(c) of the Clean Air Act of 1955, as amended (42 U.S.C. §§7401 et seq.); (g) protection of underground sources of drinking water under the Safe Drinking Water Act of 1974, as amended (P.L. 93-523); and, (h) protection of endangered species under the Endangered Species Act of 1973, as amended (P.L. 93-205).
12. Will comply with the Wild and Scenic Rivers Act of 1968 (16 U.S.C. §§1271 et seq.) related to protecting components or potential components of the national wild and scenic rivers system.
13. Will assist the awarding agency in assuring compliance with Section 106 of the National Historic Preservation Act of 1966, as amended (16 U.S.C. §470), EO 11593 (identification and protection of historic properties), and the Archaeological and Historic Preservation Act of 1974 (16 U.S.C. §§469a-1 et seq.).
14. Will comply with P.L. 93-348 regarding the protection of human subjects involved in research, development, and related activities supported by this award of assistance.
15. Will comply with the Laboratory Animal Welfare Act of 1966 (P.L. 84544, as amended, 7 U.S.C. §§2131 et seq.) pertaining to the care, handling, and treatment of warm blooded animals held for research, teaching, or other activities supported by this award of assistance.
16. Will comply with the Lead-Based Paint Poisoning Prevention Act (42 U.S.C. §§4801 et seq.) which prohibits the use of lead-based paint in construction or rehabilitation of residence structures.
17. Will cause to be performed the required financial and compliance audits in accordance with the Single Audit Act Amendments of 1996 and OMB Circular No. A-133, 'Audits of States, Local Governments, and Non-Profit Organizations.'
18. Will comply with all applicable requirements of all other Federal laws, executive orders, regulations, and policies governing this program.

SIGNATURE OF AUTHORIZED CERTIFYING OFFICIAL 	TITLE Sandra M. Dowdy Contracts and Grants Analyst
APPLICANT ORGANIZATION THE REGENTS OF THE UNIVERSITY OF CALIFORNIA	DATE SUBMITTED MAY 12 2000

Certifications Regarding Debarment, Suspension and
Other Responsibility Matters, **Drug-Free Workplace**
Requirements and Lobbying

Persons signing this form should refer to the regulations referenced below for complete instructions:

Certification Regarding Debarment, Suspension, and Other Responsibility Matters - Primary Covered Transactions - The prospective primary participant further agrees by submitting this proposal that it ~~will~~ include the clause titled, "Certification Regarding Debarment, Suspension, Ineligibility and Voluntary Exclusion - Lower Tier Covered Transaction," provided by the department or agency entering into this covered transaction, without modification, in all lower tier covered transactions and in all solicitations for lower tier covered transactions. See below for language to be used; use this form for certification and sign; or use Department of the Interior Form 1954 (DI-1954). (See Appendix A of Subpart D of 43 CFR Part 12.)

Certification Regarding Debarment, Suspension, Ineligibility and Voluntary Exclusion - Lower Tier Covered Transactions - (See Appendix B of Subpart D of 43 CFR Part 12.)

Certification Regarding Drug-Free Workplace Requirements: ~~Allegation I. (Grantees Other Than Individuals) and Allegation II. (Grantees Who are Individuals)~~ - (See Appendix C of Subpart D of 43 CFR Part 12.)

Signature on this form provides for compliance with certification requirements under 43 CFR Parts 12 and 18. The certifications shall be treated as a material representation of fact upon which reliance will be placed when the Department of the Interior determines to award the covered transaction, grant, cooperative agreement or loan.

PART A Certification Regarding Debarment, Suspension, and Other Responsibility Matters -
Primary Covered Transactions

CHECK ☐ IF THIS CERTIFICATION IS FOR A PRIMARY COVERED TRANSACTION AND IS APPLICABLE.

- (1) The prospective primary participant certifies to the best of its knowledge and belief, that it and its principals:
 - (a) ~~Are not presently~~ debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from covered transactions by any Federal department or agency;
 - (b) Have not within a three year period preceding this proposal been convicted of or had a civil judgment rendered against them for commission of fraud or a criminal offense in connection with obtaining, attempting to obtain, or performing a public (Federal, State or local) transaction or contract under a public transaction; violation of Federal or State antitrust statutes or commission of embezzlement, theft, forgery, bribery, falsification or destruction of records, making false statements, or receiving stolen property;
 - (c) ~~Are not presently indicted for or otherwise~~ criminally or civilly charged by a governmental entity (Federal, State or local) with commission of any of the offenses enumerated in paragraph (1)(b) of this certification; and
 - (d) Have not within a three year period preceding this application/proposal had one or more public transactions (Federal, State or local) terminated for cause or default.
- (2) Where the prospective primary participant is unable to certify to any of the statements in this certification, such prospective participant shall attach an explanation to this proposal.

PART B: Certification Regarding Debarment, Suspension, Ineligibility and Voluntary Exclusion -
Lower Tier Covered Transactions

CHECK ☐ IF THIS CERTIFICATION IS FOR A LOWER TIER COVERED TRANSACTION AND IS APPLICABLE.

- (1) The prospective lower tier participant certifies, by submission of this proposal, that neither it nor its principals is presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from participation in this transaction by any Federal department or agency.
- (2) Where the prospective lower tier participant is unable to certify to any of the statements in this certification, such prospective participant shall attach an explanation to this proposal.

PART C: Certification Regarding Drug-Free Workplace Requirements

CHECK ~~XX~~ IF THIS CERTIFICATION IS FOR AN APPLICANT WHO IS NOT AN INDIVIDUAL.

Alternate I. (Grantees Other Than Individuals)

A. The grantee certifies that it will or continue to provide a drug-free workplace by:

- (a) Publishing a statement notifying employees that the unlawful manufacture, distribution, dispensing, possession, or use of a controlled substance is prohibited in the grantee's workplace and specifying the actions that will be taken against employees for violation of such prohibition;
- (b) Establishing an ongoing drug-free awareness program to inform employees about--
 - (1) The dangers of drug abuse in the workplace;
 - (2) The grantee's policy of maintaining a drug-free workplace;
 - (3) Any available drug counseling, rehabilitation, and employee assistance programs; and
 - (4) The penalties that may be imposed upon employees for drug abuse violations occurring in the workplace;
- (c) Making it a requirement that each employee to be engaged in the performance of the grant be given a copy of the statement required by paragraph (a);
- (d) Notifying the employee in the statement required by paragraph (a) that, as a condition of employment under the grant, the employee will --
 - (1) Abide by the terms of the statement; and
 - (2) Notify the employer in writing of his or her conviction for a violation of a criminal drug statute occurring in the workplace no later than five calendar days after such conviction;
- (e) Notifying the agency in writing, within ten calendar days after receiving notice under subparagraph (d)(2) from an employee or otherwise receiving actual notice of such conviction. Employers of convicted employees must provide notice, including position title, to every grant officer on whose grant activity the convicted employee was working, unless the Federal agency has designated a central point for the receipt of such notices. Notice shall include the identification number(s) of each affected grant;
- (f) Taking one of the following actions, within 30 calendar days of receiving notice under subparagraph (d)(2), with respect to any employee who is so convicted --
 - (1) Taking appropriate personnel action against such an employee, up to and including termination, consistent with the requirements of the Rehabilitation Act of 1973, as amended; or
 - (2) Requiring such employee to participate satisfactorily in a drug abuse assistance or rehabilitation program approved for such purposes by a Federal, State, or local health, law enforcement, or other appropriate agency;
- (g) Making a good faith effort to continue to maintain a drug-free workplace through implementation of paragraphs (a), (b), (c), (d), (e) and (f).

B. The grantee may insert in the space provided below the site(s) for the performance of work done in connection with the specific grant:

Place of Performance (Street address, city, county, state, zip code)

University of California
One Shields Ave
Davis CA 95616

Check ☐ if there are workplaces on file that are not identified here.

PART D: Certification Regarding Drug-Free Workplace Requirements

CHECK ☐ IF THIS CERTIFICATION IS FOR AN APPLICANT WHO IS AN INDIVIDUAL.

Alternate II. (Grantees Who Are Individuals)

- (a) The grantee certifies that, as a condition of the grant, he or she will not engage in the unlawful manufacture, distribution, dispensing, possession, or use of a controlled substance in conducting any activity with the grant;
- (b) If convicted of a criminal drug offense resulting from a violation occurring during the conduct of any grant activity, he or she will report the conviction, in writing, within 10 calendar days of the conviction, to the grant officer or other designee, unless the Federal agency designates a central point for the receipt of such notices. When notice is made to such a central point, it shall include the identification number(s) of each affected grant.

DI-2010
March 1995
(This form consolidates DI-1953, DI-1954,
DI-1955, DI-1956 and DI-1963)

CHECK ☐ IF CERTIFICATION IS FOR THE AWARD OF ANY OF THE FOLLOWING AND THE AMOUNT EXCEEDS \$100,000: A FEDERAL GRANT OR COOPERATIVE AGREEMENT, SUBCONTRACT, OR SUBGRANT UNDER THE GRANT OR COOPERATIVE AGREEMENT.

CHECK ☐ IF CERTIFICATION IS FOR THE AWARD OF A FEDERAL LOAN EXCEEDING THE AMOUNT OF \$150,000, OR A SUBGRANT OR SUBCONTRACT EXCEEDING \$100,000, UNDER THE LOAN.

The undersigned certifies, to the best of his or her knowledge and belief, that:

- (1) No Federal appropriated funds have been paid or ~~will~~ be paid, by or on behalf of the undersigned, to any person for influencing or attempting to influence an officer or employee of an agency, a Member of Congress, and officer or employee of Congress, or an employee of a Member of Congress in connection with the awarding of any Federal contract, the making of any Federal grant, the making of any Federal loan, the entering into of any cooperative agreement, and the extension, continuation, renewal, amendment, or modification of any Federal contract, grant, loan, or cooperative agreement.
- (2) If any funds other than Federal appropriated funds have been paid or ~~will~~ be paid to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with this Federal contract, grant, loan, or Cooperative agreement, the undersigned shall complete and submit Standard Form-LLL, "Disclosure Form to Report Lobbying," in accordance with its instructions.
- (3) The undersigned shall require that the language of this certification be included in the award documents for all subawards at all tiers (including subcontracts, subgrants, and contracts under grants, loans, and cooperative agreements) and that all subrecipients shall certify accordingly.

This certification is a material representation of fact upon which reliance was placed ~~when~~ this transaction ~~was~~ made or entered into. Submission of this certification is a prerequisite for making or entering into this transaction imposed by Section 1352, title 31, U.S. Code. Any person who fails to file the required certification shall be subject to a civil penalty of not less than \$10,000 and not more than \$100,000 for each such failure.

As the authorized certifying official, I hereby certify that the above specified certifications are true.

SIGNATURE OF AUTHORIZED CERTIFYING OFFICIAL



TYPED NAME AND TITLE

Sandra M. Dowdy
Contracts and Grants Analyst

DATE

MAY 12 2000

APPLICATION FOR FEDERAL ASSISTANCE

OMB Approval No. 0348-0043

2. DATE SUBMITTED 5-15-00		Applicant Identifier
3. DATE RECEIVED BY STATE		State Application Identifier
4. DATE RECEIVED BY FEDERAL AGENCY		Federal Identifier

TYPE OF SUBMISSION: <input type="checkbox"/> Application <input checked="" type="checkbox"/> Construction <input type="checkbox"/> Non-Construction		PREAPPLICATION <input type="checkbox"/> Construction <input type="checkbox"/> Non-Construction
APPLICANT INFORMATION Applicant Name: Joseph J. Cech, Jr.		Organizational Unit: University of California, Davis
Address (give city, county, State, and zip code): Dept. Wildlife, Fish, and Conservation Biology University of California, Davis Davis, Yolo County, CA 95616		Name and telephone number of person to be contacted on matters involving this application (give area code): Joseph J. Cech, Jr. 530-752-3103

EMPLOYER IDENTIFICATION NUMBER (EIN): 94-6036494		7. TYPE OF APPLICANT: (enter appropriate letter in box) <input checked="" type="checkbox"/> I A. State B. County C. Municipal D. Township E. Interstate F. Intermunicipal G. Special District H. Independent School Dist. I. State Controlled Institution of Higher Learning J. Private University K. Indian Tribe L. Individual M. Profit Organization N. Other (Specify) _____
TYPE OF APPLICATION: <input checked="" type="checkbox"/> New <input type="checkbox"/> Continuation <input type="checkbox"/> Revision Revision, enter appropriate letter(s) in box(es): <input type="checkbox"/> <input type="checkbox"/> A. Increase Award B. Decrease Award C. Increase Duration D. Decrease Duration Other (specify): _____		9. NAME OF FEDERAL AGENCY: U.S. Bureau of Reclamation

CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER: TITLE: AREAS AFFECTED BY PROJECT (Cities, Counties, States, etc.): Sacramento, Solano, Marin, Yolo, Del Norte, and Humboldt Counties		11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT: Biological Assessment of Green Sturgeon in the Sacramento-San Joaquin Watershed
--	--	--

PROPOSED PROJECT Start Date: 4-1-01 Ending Date: 13-31-03		14. CONGRESSIONAL DISTRICTS OF: a. Applicant Congressional District # 3 b. Project Congressional District # 3																						
ESTIMATED FUNDING: <table border="1"> <tr> <td>Federal</td> <td>\$ 641,362</td> <td></td> </tr> <tr> <td>Applicant</td> <td>\$</td> <td></td> </tr> <tr> <td>State</td> <td>\$</td> <td></td> </tr> <tr> <td>Local</td> <td>\$</td> <td></td> </tr> <tr> <td>Other</td> <td>\$</td> <td></td> </tr> <tr> <td>Program Income</td> <td>\$</td> <td>m</td> </tr> <tr> <td>TOTAL</td> <td>\$ 641,362</td> <td></td> </tr> </table>		Federal	\$ 641,362		Applicant	\$		State	\$		Local	\$		Other	\$		Program Income	\$	m	TOTAL	\$ 641,362		16. IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS? a. YES. THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON: DATE _____ b. No. <input type="checkbox"/> PROGRAM IS NOT COVERED BY E. O. 12372 <input type="checkbox"/> OR PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW	
Federal	\$ 641,362																							
Applicant	\$																							
State	\$																							
Local	\$																							
Other	\$																							
Program Income	\$	m																						
TOTAL	\$ 641,362																							
		17. IS THE APPLICANT DELINQUENT ON ANY FEDERAL DEBT? <input type="checkbox"/> Yes If "Yes," attach an explanation. <input checked="" type="checkbox"/> No																						

TO THE BEST OF MY KNOWLEDGE AND BELIEF, ALL DATA IN THIS APPLICATION/PREAPPLICATION ARE TRUE AND CORRECT, THE DOCUMENT HAS BEEN DULY AUTHORIZED BY THE GOVERNING BODY OF THE APPLICANT AND THE APPLICANT WILL COMPLY WITH THE ATTACHED ASSURANCES IF THE ASSISTANCE IS AWARDED.

Type Name of Authorized Representative	b. Title Sandra M. Dowdy Contracts and Grants Analyst	c. Telephone Number
Signature of Authorized Representative Sandra M. Dowdy		d. Date Signed MAY 12 2000

BUDGET INFORMATION - Non-Construction Programs

SECTION A - BUDGET SUMMARY

Grant Program Function or Activity (a)	Catalog of Federal Domestic Assistance Number (b)	Estimated Unobligated Funds			New or Revised Budget	
		Federal (c)	Non-Federal (d)	Federal (e)	Non-Federal (f)	Total (g)
1. Task 1, Fish Env. Requirements		\$	\$	\$170,256	0	\$170,256
2. Task 2, Fish Reproduction				105,245	0	105,245
3. Task 3, Genetics				112,272	0	112,272
4a. Task 4, Telemetry, BML+SFSU				179,751	0	179,751
4b. Task 5, Field Sampling, DFG				62,543	0	62,543
4c. Task 6, Project Management				2,235	0	2,235
5. Totals		\$	\$	\$632,482	0	\$632,482

SECTION B - BUDGET CATEGORIES

6. Object Class Categories	GRANT PROGRAM, FUNCTION OR ACTIVITY						Total (5)
	(1)	(2)	(3)	(4a)	(4b)	(4c)	
a. Personnel	\$80,000	\$47,959	\$50,430	\$87,321	\$24,670	\$1,000	\$291,380
b. Fringe Benefits	1,400	11,510	12,608	10,870	4,934	18	41,340
c. Travel	4,000	2,000	1,200	1,600	0	0	8,800
d. Equipment	0	0	4,000	5,060	0	0	9,060
e. Supplies	18,000	10,000	12,000	22,398	5,820	500	68,718
f. Contractual (DFG + SFSU Overhead)	0	0	0	8,492	7,050	0	15,542
g. Construction	0	0	0	0	0	0	0
h. Other (Student Fee Remissions)	18,000	0	0	9,750	0	0	27,750
i. Total Direct Charges (sum of 6a-6h)	121,400	71,469	80,238	145,491	42,474	1,518	462,590
j. Indirect Charges	48,856	33,776	36,034	39,320	20,069	717	178,772
k. TOTALS (sum of 6i and 6j)	\$170,256	\$105,245	\$116,272	\$184,811	\$62,543	\$2,235	\$641,362
7. Program Income	\$ 0	\$ 0	0	0	\$ 0	\$ 0	\$ 0

SECTION C - NON FEDERAL RESOURCES

(a) Grant Program	(b) Applicant	(c) State	(d) Other Sources	(e) TOTALS
8. Task 1, Fish Env. Req.	\$12,739	0	0	\$12,739
9. Task 2, Fish Reprod.	\$12,739	0	0	\$12,739
10. Task 3, Genetics	0	0	0	0
11a. Task 4, Telem. (BML + SFSU)	0	0	0	0
11b. Task 5, Field (CDFG)	0	60,000	0	60,000
11c. Task 6, Project Mgmt.	0	0	0	0
12. TOTAL (sum of lines 8 - 11)	\$25,478	\$60,000	0	\$85,478

SECTION D - FORECASTED CASH NEEDS

	Total for 1st Year	1st Quarter	2nd Quarter	3rd Quarter	4th Quarter
13. Federal	\$321,375	\$87,139	\$78,079	\$78,079	\$78,078
14. NonFederal	\$42,739	\$10,685	\$10,685	\$10,685	\$10,684
15. TOTAL (sum of lines 13 and 14)	\$364,114	\$97,824	\$88,764	\$88,764	\$88,762

SECTION E - BUDGET ESTIMATES OF FEDERAL FUNDS NEEDED FOR BALANCE OF THE PROJECT

(a) Grant Program	FUTURE FUNDING PERIODS (Years)			
	(b) First	(c) Second	(d) Third	(e) Fourth
16 Task 1, Fish Environmental Requirements	\$21,379	\$21,379	\$21,379	\$21,379
17 Tasks 2 + 3 (Fish Reproduction + Genetics)	27,638	27,818	27,818	27,818
18 Task 4 (Telemetry)	22,707	22,706	22,706	22,706
19 Tasks 5 + 6 (Field Sampling + Project Mgmt.)	8,139	8,139	8,138	8,138
20. TOTAL (sum of lines 16-19)	\$79,863	\$80,042	\$80,041	\$80,041

SECTION F - OTHER BUDGET INFORMATION

21. Direct Charges:	\$462,590	22. Indirect Charges:	178,772
23. Remarks:			

DEPARTMENT OF FISH AND GAME
Central Valley Bay-Delta Branch
4001 North Wilson Way
Stockton, California 95205-2486



May 8, 2000

Dr. Joseph J. Cech, Jr.
Department of Wildlife, Fish, and Conservation Biology
University of California
Davis, CA 95616

Dear Dr. Cech

Subject to all applicable State of California and University of California contracting requirements, we agree to provide subcontracted services (from 4/1/01 to **3/31/03**) to the University of California, Davis, regarding the proposed CALFED contract entitled "Biological Assessment of Green Sturgeon in the Sacramento-San Joaquin Watershed." These services would consist of assistance with fish capture for fish tracking activities as described in the project proposal (Task 5). In return for these services, the California Department of Fish and Game will be paid \$21,237 per year for two years (through UC Davis), as shown in the budget section of the project proposal (Task 4). If you have questions concerning this matter, please contact the principal investigator, Mr. David Kohlhorst at 209-948-7080 or dkohlhor@delta.dfg.ca.gov.

Sincerely,

A stylized signature consisting of a large '@' symbol followed by a horizontal line.

Al Caracco
Operations Manager
Central Valley Bay-Delta Branch

cc: Dr. Perry Herrgesell
Mr. David Kohlhorst



San Francisco State University
1600 Holloway Avenue
San Francisco, California 94132

Office of Research and
Sponsored Programs
415/338-2231

May 11, 2000

Joseph J. Cech, Jr.
Department of Wildlife, Fish and Conservation Biology
University of California
Davis, CA 95616

Dear Dr. Cech:

I agree to provide subcontracted services (from 4/1/01 to 3/31/03) to The University of California, Davis, regarding the proposed CALFED contract entitled "Biological Assessment of Green Sturgeon in the Sacramento-San Joaquin Watershed". These services would consist of assistance with Fish tracking activities as described in the project proposal (Task 4). In return for these services, San Francisco State University will be paid \$12,570 per year for two years (total of the student salary + SFSU overhead on this) from CALFED (through UC Davis), as shown in the budget section of the project proposal (Task 4).

Sincerely,

A handwritten signature in black ink, appearing to read "Carlos E. Crocker".

Carlos E. Crocker, Ph.D.
SFSU Investigator

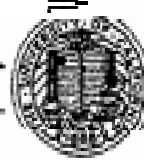
A handwritten signature in black ink, appearing to read "Paul J. Fonteyn".

Paul J. Fonteyn, Associate Vice President
Research and Sponsored Programs

PJF/vrh

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COLLEGE OF AGRICULTURAL AND
ENVIRONMENTAL SCIENCES
AGRICULTURAL EXPERIMENT STATION
COOPERATIVE EXTENSION

DEPARTMENT OF WILDLIFE, FISH AND CONSERVATION BIOLOGY
ONE SHIELDS AVENUE
DAVIS, CALIFORNIA 95616-8751
FAX (530)752-4154

May 12, 2000

Yolo County Board of Supervisors
625 Court
Woodland, CA 95695-3448

Dear Sir or Madam,

This letter to inform you that I have submitted a proposal entitled "Biological Assessment of Green Sturgeon in the Sacramento-SanJoaquin Watershed" to the CALFED Ecosystem Restoration Program. ~~Part~~ of the work described in the proposal will be conducted at the University of California, Davis, in Yolo County.

Sincerely,

A handwritten signature in dark ink, appearing to read "Joseph J. Cech, Jr.", is written over a light blue rectangular background.

Joseph J. Cech, Jr.
Professor

cc: CALFED Bay/Delta Program

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DEPARTMENT OF WILDLIFE, FISH AND CONSERVATION BIOLOGY
ONE SHIELDS AVENUE
DAW, CALIFORNIA 95616.8751
FAX (530) 7524154

May 12, 2000

Sacramento County Board of Supervisors
700 H Street
Sacramento, CA 95814

Dear Sir or Madam,

This letter to inform you that I have submitted a proposal entitled "Biological Assessment of Green Sturgeon in the Sacramento-Sau Joaquin Watershed" to the CALFED Ecosystem Restoration Program. Part of the work described in the proposal will be conducted in Sutter county.

Sincerely,

A handwritten signature in dark ink, appearing to read "Joseph J. Cech, Jr.", is written over a light-colored rectangular background.

Joseph J. Cech, Jr.
Professor

cc: CALFED Bay/Delta Program

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DEPARTMENT OF WILDLIFE, FISH AND CONSERVATION BIOLOGY
ONE SHIELDS AVENUE
DAVIS, CALIFORNIA 95616-8751
FAX (530) 7524154

May 12, 2000

Solano County Board of Supervisors
580 Texas Street
Fairfield, CA 94533

Dear Sir or Madam,

This letter to inform you that I have submitted a proposal entitled "Biological Assessment of Green Sturgeon in the Sacramento-San Joaquin Watershed" to the CALFED Ecosystem Restoration Program. Part of the work described in the proposal will be conducted in Sutter county.

Sincerely,

A handwritten signature in dark ink, appearing to read "Joseph J. Cech, Jr.", written over a light-colored rectangular background.

Joseph J. Cech, Jr.
Professor

cc: CALFED Bay/Delta Program

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DEPARTMENT OF WILDLIFE, FISH AND CONSERVATION BIOLOGY
ONE SHIELDS AVENUE
DAVIS, CALIFORNIA 95616-8751
FAX (530) 752-4154

May 12, 2000

Marin County Board of Supervisors
3501 Civic Center Drive
Room 329
San Rafael, CA 94903

Dear Sir or Madam,

This letter to inform you that I have submitted a proposal entitled "Biological Assessment of Green Sturgeon in the Sacramento-San Joaquin Watershed" to the CALFED Ecosystem Restoration Program. Part of the work described in the proposal will be conducted in Sutter County.

Sincerely,

A handwritten signature in dark ink, appearing to read "Joseph J. Cech, Jr.".

Joseph J. Cech, Jr.
Professor

cc: CALFED Bay/Delta Program

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DEPARTMENT OF WILDLIFE, FISH AND CONSERVATION BIOLOGY
ONE SHIELDS AVENUE
DAVIS, CALIFORNIA 95616-8751
FAX (530) 752-4154

May 12, 2000

Del Norte County Board of Supervisors
583 G Street
Suite #1
Crescent City, CA 95531

Dear Sir or Madam,

This letter to inform you that I have submitted a proposal entitled "Biological Assessment of Green Sturgeon in the Sacramento-San Joaquin Watershed" to the CALFED Ecosystem Restoration Program. Part of the work described in the proposal will be conducted in Sutter county.

Sincerely,

A handwritten signature in black ink, reading "Joseph J. Cech, Jr." in a cursive script.

Joseph J. Cech, Jr.
Professor

cc: CALFED Bay/Delta Program

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DEPARTMENT OF WILDLIFE, FISH AND CONSERVATION BIOLOGY
ONE SHIELDS AVENUE
DAVIS, CALIFORNIA 95616-8751
FAX (530) 752-4154

May 12, 2000

Humboldt County Board of Supervisors
825 5th Street
Eureka, CA 95501-1153

Dear ~~Sr~~ or Madam,

This letter to inform you that I have submitted a proposal entitled 'Biological Assessment of Green Sturgeon in the Sacramento-San Joaquin Watershed' to the CALFED Ecosystem Restoration Program. Part of the work described in the proposal will be conducted in Sutter county;

Sincerely,

A handwritten signature in dark ink, appearing to read 'Joseph J. Cech, Jr.', is written over a light blue rectangular background.

Joseph J. Cech, Jr.
Professor

cc: CALFED Bay/Delta Program

Environmental Compliance Checklist

All applicants must fill out **this** Environmental Compliance Checklist. Applications must contain answers to the following questions to be responsive **and** to be considered for funding. **Failure to answer these questions and include them with the application will result in the application being considered nonresponsive and not considered for funding.**

1. Do any of the actions included in the proposal require compliance with either the California Environmental Quality Act (CEQA), the National Environmental Policy Act (NEPA), or both?

YES

X
NO

2. If you answered yes to # 1, identify the lead governmental agency for CEQA/NEPA compliance.

Lead Agency

3. If you answered no to # 1, explain why CEQA/NEPA compliance is not required for the actions in the proposal.

Project consists of research, and does not include environmental modifications or alterations. Fish specimens will be collected under existing or pending scientific collecting permits/agreements.

4. If CEQA/NEPA compliance is required, describe how the project will comply with either or both of these laws. Describe where the project is in the compliance process and the expected date of completion.

5. Will the applicant require access across public or private property that the applicant does not own to accomplish the activities in the proposal?

YES

X
NO

If yes, the applicant must attach written permission for access from the relevant property owner(s). Failure to include written permission for access may result in disqualification of the proposal during the review process. Research and monitoring field projects for which specific field locations have not been identified will be required to provide access needs and permission for access with 30 days of notification of approval.

6. Please indicate what permits or other approvals may be required for the activities contained in your proposal. Check all boxes that apply.

LOCAL

Conditional use permit

Variance

Subdivision Map Act approval

Grading permit

General plan amendment

Specific plan approval

Rezone

Williamson Act Contract
cancellation

Other _____
(please specify)

None required.

STATE

CESA Compliance

Streambed alteration permit

CWA § 401 certification

Coastal development permit

Reclamation Board approval

Notification

Other _____
(please specify)

None required

FEDERAL

ESA Consultation

Rivers & Harbors Act permit

CWA § 404 permit

Other _____
(please specify)

None required

(CDFG)

(CDFG)

(RWQCB)

(Coastal Commission/BCDC)

(DPC, BCDC)

(USFWS)

(ACOE)

(ACOE)

DPC = Delta Protection Commission
CWA = Clean Water Act
CESA = California Endangered Species Act
USFWS = U.S. Fish and Wildlife Service
ACOE = U.S. Army Corps of Engineers

ESA = Endangered Species Act
CDFG = California Department of Fish and Game
RWQCB = Regional Water Quality Control Board
BCDC = Bay Conservation and Development Comm.

Land Use Checklist

All applicants must fill out this Land Use Checklist for their proposal. Applications must contain answers to the following questions to be responsive and to be considered for funding. *Failure to answer these questions and include them with the application will result in the application being considered nonresponsive and not considered for funding.*

1. Do the actions in the proposal involve physical changes to the land (i.e. grading, planting vegetation, or breaching levees) or restrictions in land use (i.e. conservation easement or placement of land in a wildlife refuge)?

YES

X
NO

2. If NO to # 1, explain what type of actions are involved in the proposal (i.e., research only, planning only).

Project involves only research.

3. If YES to # 1, what is the proposed land use change or restriction under the proposal?

4. If YES to # 1, is the land currently under a Williamson Act contract?

YES

NO

If YES to # 1, answer the following:

Current land use

Current zoning

Current general plan designation

6. If YES to #1, is the land classified as Prime Farmland, Farmland of Statewide Importance or Unique Farmland on the Department of Conservation Important Farmland Maps?

YES

NO

DON'T KNOW

7. If YES to # 1, how many acres of land will be subject to physical change or land use restrictions under the proposal?

8. If YES to # 1, is the property currently being commercially farmed or grazed?

YES

NO

9. If YES to #8, what are

the number of employees/acre
the total number of employees

10. Will the applicant acquire any interest in land under the proposal (fee title **or** a conservation easement)?

YES

X.
NO

11. What entity/organization will hold the interest? _____

12. If **YES** to # 10, answer the following:

Total number of acres to be acquired under proposal _____

Number of acres to be acquired in fee _____

Number of acres to be subject to conservation easement _____

13. For all proposals involving physical changes to the land **or** restriction in land use, describe what entity or organization will:

manage the property _____

provide operations and maintenance services _____

conduct monitoring _____

14. For land acquisitions (fee title **or** easements), will existing water rights also be acquired?

YES

NO

15. Does the applicant propose any modifications to the water right **or** change in the delivery of the water?

YES

X.
NO

16. If **YES** to # 15, describe _____